LSAMP AND *NORE1* DOWN-REGULATION IN CLEAR CELL RENAL CELL CARCINOMAS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention in the field of genetics and medicine relates to the under-expression of two genes, *LSAMP* and *NORE1* in clear cell renal cell carcinoma (CC-RCC) tissue and exploitation of this property in methods for detecting or treating this type of cancer.

Description of the Background Art

Renal carcinoma is known to have different histological types, with distinct genetic profiles (Storkel *et al.*, 1997). Worldwide, approximately 150,000 people are diagnosed with renal carcinoma, resulting in 78,000 deaths annually (Zbar *et al.*, 2002). The most common type is clear cell renal cell carcinoma (CC-RCC). Studies of familial CC-RCC have led to the identification of important tumor suppressor genes such as *VHL* (Latif *et al.*, 1993). Recently, position cloning also resulted in the discovery of other kidney cancer-related genes *BHD*, *FH*, and *HRPT2* (Nickerson *et al.*, 2002; Tomlinson *et al.*, 2002; Carpten *et al.*, 2002). While hereditary CC-RCCs are mainly attributed to *VHL* mutations, there are known CC-RCC families and a significant proportion of sporadic CC-RCCs that are not associated with the *VHL* (Teh *et al.*, 1997; Woodward *et al.*, 2000), thus pointing to the existence of other CC-RCC-related genes. Since some CC-RCC families are associated with balanced chromosomal translocations, the translocation breakpoint-spanning genes are likely CC-RCC-related candidate genes. The first CC-RCC family with a balanced chromosomal translocation t(3;8)(p14;q24) was described by Cohen *et al.* (1979). To date, at least eight such hereditary CC-RCC-related chromosomal translocation families have been reported (Cohen *et al.*, 1979; Kovacs *et al.*, 1988; Kovacs *et al.*, 1989; Koolen *et al.*, 1998; van Kessel *et al.*, 2001; Podolski *et al.*, 2001; Kanayama *et al.*, 2001). Interestingly, translocation in all these CC-RCC families is linked to chromosome 3, making constitutional chromosome 3 translocation a predisposing factor (vas Kessel *et al.*, 1999; Bodmer *et al.*, 1998; Bodmer *et al.*, 2002c). The subsequent observation of the loss of translocation derivative chromosome 3 (der(3) chromosome) and somatic *VHL* mutations in a proportion of familial tumors led to the proposal of a three-step model of CC-RCC tumorigenesis (Schmidt *et al.*, 1995; Bodmer *et al.*, 1998; Bodmer *et al.*, 2002c): initial constitutional chromosome 3 translocation, subsequent somatic loss of the der(3) chromosome

leading to the loss of a copy of *VHL*, and a third hit in the form of random somatic mutation in the second *VHL* allele. However, loss of the der(3) chromosome was observed only in a subset of the examined samples. Most of the analyzed familial tumors with loss of the der(3) did not carry *VHL* mutations. Furthermore, neither der(3) loss nor *VHL* mutations were observed in several tumor biopsies in the affected families (Eleveld *et al.*, 2001; Bodmer *et al.*, 2002b). These observations suggest that the breakpoint-spanning genes in the familial RCC-associated chromosome 3 translocations are also likely implicated in RCC tumorigenesis or act synergistically in the above model in the form of genetic and/or epigenetic alternations.

Analysis of the constitutional t(3;8)(p14;q24) translocation associated with familial CC-RCC led to the identification and extensive investigation of the breakpoint-spanning gene *FHIT* (fragile histidine triad) on 3p14 (Ohta *et al.*, 1996). *FHIT* is thought to be a putative tumor suppressor gene, and aberrant *FHIT* transcripts and *FHIT* genomic lesions were observed in a variety of primary tumors and tumor-derived cell lines (Ohta *et al.*, 1996; Siprashvili *et al.*, 1997; Druck *et al.*, 1997). The partner breakpoint-spanning gene *TRC8* on the chromosome 8 shows high homology to the *Drosophila* patched (*PTCH*) gene and probably also functions as a tumor suppressor (Gemmill *et al.*, 2002). Also, another two breakpoint-spanning genes, *DIRC1* on chromosome 2q33 and *DIRC2* on 3q21, disrupted respectively in t(2;3)(q33;q21) and t(2;3)(q35;q21) breakpoints, have been identified (Druck *et al.*, 2001; Bodmer *et al.*, 2002a). The role of these genes in CC-RCC tumorigenesis remains to be determined.

The present inventors describe here the positional cloning of the t(1;3)(q32.1;q13.3) chromosomal breakpoints and the identification of two breakpoint-spanning genes, *LSAMP* on 3q13.3 and *NORE1* on 1q32.1, in a previously reported Japanese hereditary CC-RCC family (Kanayama *et al.*, 2001). *LSAMP* (limbic-system-associated membrane protein gene) encodes a neuronal surface glycoprotein that belongs to the IgLONs (immunoglobulin LSAMP, OPCML/OBCAM, and neurotrimin) family and is distributed in cortical and subcortical regions of the limbic system (Pimenta *et al.*, 1996). To date, very little is known about *LSAMP* and its biological role remains unclear. However, its family partner gene *OPCML/OBCAM* on 11q25 was recently found to be epigenetically inactivated and was regarded as a candidate TSG in epithelial ovarian cancer (Sellar *et al.*, 2003). *NORE1* was recently identified as a homolog of the tumor suppressor gene *RASSF1* at 3p21.3, which is frequently inactivated via promoter hypermethylation in a variety of human tumors (Dammann *et al.*, 2000; Tommasi *et al.*, 2002). The mouse counterpart Nore1 is a Ras effector (Vavvas *et al.*, 1998).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 and its subparts show mapping of the of the t(1;3) breakpoints on chromosomes 1q32.1 and 3q13.3 by FISH.

Fig. 1A-1 shows the construction of a contig of nine BAC clones in a 3.6-cM region of 1q32.1 (left panel, labeled "1q"). The RP11-54L22 was first found to span the breakpoint by FISH split assay. Overlapped BAC clones (CTD-2245C1, -2321B11, and -2278G17) also showed split signals and confine the der(1) breakpoint region to about 30 kb (dotted box).

Fig. 1A-2 shows the similar establishment of a contig of ten BAC clones within a 5-cM region of 3q13.3 (right panel, labeled "3q"). The breakpoint was found within RP11-281N16 and was further mapped to a about 30-kb region (dotted box) using overlapping clones (RP11-149B11, CTD-2246M24, and -2514L8).

Fig. 1B shows fine mapping of the 1q32.1 and 3q13.3 breakpoints by Southern blot analysis and restriction mapping. Nine specific DNA probes (4-10 kb) flanking the 1q breakpoint were synthesized by long-range PCR with specific primers from known-sequence RP11-54L22 and -281N16. Southern blot analyses showed that a 5.6-kb 1q-p4 probe (**Fig. 1B-1** - left panel, labeled RP11-54L22 clone") and a 6.2-kb 3q-p2 probe (**Fig. 1B-2** - right panel, labeled "RP11-281N16 clone") span the respective 1q32.1 and 3q13.31 breakpoints, which narrowed both breakpoint regions to approximately 6 kb. Restriction mapping refined the 1q and the 3q breakpoints to about 1.5-kb (Fig. 1B-1, left panel) and 2-kb regions, respectively (Fig. 1B-2, right panel).

Fig. 1C shows representative Southern-blot analyses from both chromosomes showing distinct aberrant bands (indicated by arrowheads) after restriction digestion. DNA from two normal controls (N1, N2) and two patients (FRCC3 and FRCC5) were completely digested and subjected to DNA hybridization analysis. **Fig. 1C-1** shows the Southern blot from chromosome 1q32.1; **Fig. 1C-2** shows the Southern blot from chromosome 3q13.3.

Figure 2 shows the cloning of both der(1) (1q32.1) and der(3) (3q13.31) breakpoints through long-range PCR and DNA sequencing.

Fig. 2A shows amplification of der(1) and der(3) breakpoints via long-range PCR. A 2.15-kb der(1) breakpoint fragment (der(1)-BP) and a 3.25-kb der(3) breakpoint fragment (der(3)-BP) were amplified. The breakpoint fragments were sequenced and are shown in the lowest boxes. The normal sequences around the breakpoints on 1q32.1 and 3q13.31 are also

shown for comparison. The uppercase sequences are from 1q32.1 and the lowercase sequences are from 3q13.31. The sequences in red on 3q13.31 are deleted from the breakpoints.

Fig. 2B is a schematic illustration of the identification of breakpoint-spanning genes. The translocation breakpoints occur within intron 2 of both breakpoint-spanning genes *LSAMP* at 1q32.1 and *NORE1* at 3q13.31, which is accompanied by loss of 52 or 54 bp (red sequences in panel A) from *LSAMP* and of 2 or 0 bp from *NORE1*. An insertion of nucleotide G (ins G) in the breakpoint junction and a loss of 2 bp (delTG) in *LSAMP* in the distal part of breakpoint were also observed. *NORE1* has two isoforms, *NORE1A* and *NORE1B*. *LSAMP* contains seven exons and sits in the reverse strand of chromosome 3.

Figure 3 shows lower expression and promoter methylation of *LSAMP* and *NORE1A* in RCC cell lines and sporadic RCC tumors.

Fig. 3A shows that the expression of LSAMP (**Fig. 3A-1**) and NORE1A (**Fig. 3A-3**) in nine RCC cell lines and sporadic tumors (LSAMP; 0.06 ± 0.06 for cell lines and 0.05 ± 0.07 for tumors; NORE1A: 0.19 ± 0.10 for cell lines and 0.27 ± 0.12 for tumors) is significantly lower than that in nine normal kidney tissues (LSAMP; 0.77 ± 0.28; NORE1A; 1.03 ± 0.53) using real-time PCR assay (*t*-test of SSPS, $p < 0.001$). **Fig. 3A-2** and **Fig. 3A-4** (Right panels) show eight RCC cell lines which were demethylated using 5-aza-CdR; the expression of both *LSAMP* (Fig. 3A-2) and *NORE1A* (Fig. 3A-4) was significantly increased in each line (LSAMP; untreated, 0.35 ± 0.23; 5-aza-CdR, 1.02 ± 0.33; NORE1; untreated, 0.03 ± 0.02; 5-aza-CdR, 0.24 ± 0.10) (*t*-test of SSPS, $p < 0.001$).

Fig. 3B shows methylation analysis of the *LSAMP* promoter. Bisulfite-treated DNA from 53 matched pairs of human CC-RCC tumors and normal DNA samples, 9 RCC cell lines, 2 t(1;3)-positive lymphoblastoid cell lines, and 2 control lymphoblastoid cell lines (NC1 and NC2) were amplified and digested with *Hha*I. The *LSAMP* promoter (540 bp) contains 28 CpG islands. The analysed 231-bp fragment of the *LSAMP* promoter contains one *Hha*I site and digestion leads to fragments of 162 bp and 69 bp. Representative aberrant methylation of the *LSAMP* promoter in sporadic and familial CC-RCC samples and in RCC cell lines are shown.

Fig. 3C shows methylation analysis of the *NORE1A* promoter by restriction digestion with *Taq*I in the same cohort of samples. The examined 335 bp of the promoter contains 35 CpG sequences. The methylated fragment contains two *Taq*I sites and digestion results in bands of 202, 123, and 10 bp. The sizes of molecular weight markers (M) are shown on the left. N, normal kidney sample; T, RCC.

Figure 4 shows suppression of LSAMP, NORE1A, and Norel re-expression on cell proliferation characteristics.

Fig. 4A shows re-expression and localization of EGFP-LSAMP, -NORE1A, and -Norel fusion protein 2 h after microinjection or 24 h after lipid-mediated transfection of *pEGFP-LSAMP, -NORE1A,* and *-Norel* plasmids.

Fig. 42B shows a growth inhibition assay. A-498/Caki-1 cells were microinjected with *pEGFP-LSAMP, -NORE1A, -Norel,* or *pEGFP-C1/-N1* vector (negative control). Cell proliferation analysis was performed 2 h after microinjection. Cells were counted at the indicated times. The "proliferation index" on the y-axis represents the number of cells counted at those times divided by the number of cells counted 2 h after injection.

Figure 5 and its subparts show cytogenetic analysis of the t(1q32.1;3q13.3). **Fig. 5A** shows G-banding and spectral karyotyping analysis. The breakpoints were marked with arrows.

Fig. 5B shows representative results of FISH with the BAC clone probes on t(1;3) breakpoint region. **Fig. 5B-1** (Left panel) shows FISH with BAC probe CTD-2321B11 (red signal): a split signal was observed in der(3). **Fig. 5B-2** (Right panel) shows FISH with probe RP11-281N16: split signals (red) equally appeared on both der(1) and der(3). A 1q subtelomeric PAC probe 160H23 (green signal) was used as a control in all the FISH experiments.

Figure 6 and its subparts show two *NORE1* alterations identified in sporadic tumors T31 and T24. **Fig. 6A** shows that the codon 189 GTG(Val) was replaced by ATG(Met) in T31. It was also found in 5% of the control chromosomes tested. **Fig. 6B** shows that the codon 248 CGG(Arg) was replaced by CAG(Gln) in T24, which has not been detected in control samples. In *NORE1B*, the affected codon number is 95.

Figure 7 shows nuclear localization and growth suppression analysis of Norel. **Fig. 7A** demonstrates that EGFP-Norel is predominantly nuclear in Caki-1 cells (RCC cell line), by lipid-mediated transfection using a *pEGFP-Norel* plasmid and LIPOFECTAMINE 2000 reagent (Invitrogen). EGFP expressed from the empty vector *pEGFP-C1* was both nuclear and cytoplasmic. **Fig. 7B** shows that subcellular fractionation and subsequent Western analysis further indicated that the majority of Norel is localized to the nucleus, while a lesser amount appears at the plasma membrane. **Fig. 7C** shows that induction of Norel inhibits the growth of 293-T cells. The 293-T cells were transfected with *pIND(SP1)-Norel* or an empty vector and selected in hygromycin for three weeks. Each point on the growth curve represents the mean of

three individual cell count determinations. **Fig. 7D** shows western analysis of Norel expression; V, vector-transfected cells; M, Norel/MaxP1-transfected cells.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The inventors demonstrate here that the limbic-system-associated membrane protein (*LSAMP*) gene and the *NORE1* gene are the breakpoint-spanning genes in a familial clear cell renal cell carcinoma (CC-RCC), and that the expression of these genes is down-regulated in RCC cell lines and sporadic CC-RCCs. Furthermore, expression of LSAMP and NORE1A proteins in CC-RCC cell lines is shown to inhibit cell proliferation. Diagnostic and treatment methods based on the above observations are described below.

This invention includes a method for detecting the presence of, or a predisposition (susceptibility) to, a cancer (*e.g.*, CC-RCC) in a subject, comprising detecting, measuring the amount of, or quantitating LSAMP and/or NORE1 gene expression in a sample from the subject, compared to a baseline level of expression, wherein a reduction in the expression of one or both of the genes compared to the baseline level indicates that the subject suffers from, or has a predisposition to, the cancer.

As used herein, a "baseline value" or "baseline amount" includes the amount of expression of an LSAMP or a NORE1 gene in normal tissue, such as from a "pool" of normal subjects who do not suffer from, or who do not exhibit a predisposition to, the cancer. This value can be determined at the same time as the level in a sample from the subject being studied, or it can be available in a reference database such as a reference standard or a generic database.

The expression may be at the level of RNA transcription which can be detected by various means including quantitative hybridization to a suitable probe, or at the level of protein translation, for example by determining the activity of, or the presence of, the protein, using conventional procedures including an immunoassay. Methods for detecting, measuring or quantitating either the RNA or the protein gene product are conventional and routine.

In the following description, reference will be made to various methodologies known to those of skill in the art of immunology, cell biology, and molecular biology. Publications and other materials setting forth such known methodologies to which reference is made are incorporated herein by reference in their entireties as though set forth in full. Standard reference works setting forth the general principles of immunology include A.K. Abbas *et al.*, *Cellular and Molecular Immunology* (Fourth Ed.), W.B. Saunders Co., Philadelphia, 2000; C.A. Janeway

et al., *Immunobiology. The Immune System in Health and Disease*, Fourth ed., Garland Publishing Co., New York, 1999; Roitt, I. *et al.*, *Immunology*, (current ed.) C.V. Mosby Co., St. Louis, MO (1999); Klein, J., *Immunology*, Blackwell Scientific Publications, Inc., Cambridge, MA, (1990).

Monoclonal antibodies (mAbs) and methods for their production and use are described in Kohler and Milstein, *Nature 256*:495-497 (1975); U.S. Patent No. 4,376,110; Harlow, E. *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988); *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, New York, NY (1980); H. Zola *et al.*, in *Monoclonal Hybridoma Antibodies: Techniques and Applications,* CRC Press, 1982)); (Kozbor *et al.*, 1983, *Immunol. Today 4*:72 (the human B-cell hybridoma technique), and Cole, *et al.*, 1985, In: *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (the EBV-hybridoma technique to produce human mAbs).

Interspecies chimeric antibodies are described, for example, in Cabilly *et al.*, U.S. Patents 4,816,567 (3/28/89) and 6,331,415 (12/18/01);; Morrison *et al.*, US Patent 5,807,715 (9/15/98) and Eur. Patent Pub. EP173494 (3/5/86); Taniguchi *et al.*, Eur. Patent Pub. EP171496 (2/19/86); Neuberger *et al.*, PCT Pub. WO86/01533 (3/13/86); Robinson *et al.*, PCT Pub. WO 8702671 (5/7/87); Cabilly *et al.*, *Proc. Natl. Acad. Sci. USA 81*:3273-3277 (1984); Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne *et al.*, *Nature 312*:643-646 (1984); Morrison, *Science, 229*:1202-1207 (1985); Neuberger *et al., Nature 314*:268-270 (1985); Takeda *et al.*, *Nature 314*:452-454 (1985); Oi *et al.*, *BioTechniques 4*:214 (1986); Sun *et al.*, *Proc. Natl. Acad. Sci. USA 84*:214-218 (1987); Liu *et al.*, *J. Immunol. 139*:3521-3526 (1987); Better, M., *et al.*, *Science 240*:1041-1043 (May 20, 1988); and Horwitz, A. H., *et al.*, *Proc. Natl. Acad. Sci. USA 85*:8676-8682 (1988)).

Single chain antibodies (scFv) are described, for example, in Skerra, A. *et al.* (1988) *Science*, 240: 1038-1041; Pluckthun, A. *et al.* (1989) *Methods Enzymol.* 178: 497-515; Winter, G. *et al.* (1991) *Nature*, 349: 293-299); Bird *et al.*, (1988) *Science* 242:423; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879; Jost CR *et al,. J Biol Chem.* 1994 *269*:26267-26273.U.S. Patents No. 4,704,692, 4,853,871, 4,946,778, 5,260,203, 5,455,030; and Jost CR *et al. J Biol Chem.* 1994 *269*:26267-26273.

Immunoassay methods are also described in Coligan, J.E. *et al.*, eds., *Current Protocols in Immunology*, Sec. 2.4.1, Wiley-Interscience, New York, 1992 or current edition); Butt, W.R. (ed.) *Practical Immunoassay: The State of the Art*, Dekker, New York, 1984; Bizollon, Ch. A.,

ed., *Monoclonal Antibodies and New Trends in Immunoassays*, Elsevier, New York, 1984; Butler, J.E., ELISA (Chapter 29), In: van Oss, C.J. *et al.*, (eds), *IMMUNOCHEMISTRY*, Marcel Dekker, Inc., New York, 1994, pp. 759-803; Butler, J.E. (ed.), *Immunochemistry of Solid-Phase Immunoassay*, CRC Press, Boca Raton, 1991; Weintraub, B., *Principles of Radioimmunoassays*, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986; Work, T.S. *et al.*, *Laboratory Techniques and Biochemistry in Molecular Biology*, North Holland Publishing Company, NY, (1978) (Chapter by Chard, T., "An Introduction to Radioimmune Assay and Related Techniques").

In a preferred embodiment, the presence or amount of LSAMP and/or NORE1 protein in a cell is detected by binding proteins in the sample to a detectably labeled antibody that is specific for an LSAMP or a NORE1 protein. An antibody "specific" for a polypeptide means that the antibody recognizes a defined sequence of amino acids, or epitope, either present in the full length polypeptide, or in a peptide fragment thereof.

Any of a variety of antibodies can be used in such methods. Such antibodies include, polyclonal, monoclonal (mAbs), recombinant, humanized or partially humanized, single chain (scFv), Fab, and fragments thereof. The antibodies can be of any isotype, such as IgM, various IgG isotypes such as IgG_1' IgG_{2a}, *etc.*, and they can be from any animal species that produces antibodies, including goat, rabbit, mouse, chicken or the like.

Antibodies are prepared according to conventional methods, which are well known. See, references cited above. Methods of preparing humanized or partially humanized antibodies, and antibody fragments, and methods of purifying antibodies, are conventional (*supra*).

For preparation of mAbs, any technique that provides mAbs produced by cell lines in continuous culture can be used (*supra*). Techniques described for the production of single chain antibodies (*supra*) can be adapted to produce scFv antibodies to polypeptide products of this invention.

Transgenic animals may be used to express partially or fully humanized antibodies to immunogenic polypeptide products of this invention.

Other specific binding partners, such as, *e.g.*, aptamers and peptide nucleic aces (PNA), may be used in place of antibodies.

The sample to be assayed in a method of the invention may be any suitable cell or tissue, or extract thereof. A sample of a body fluid such as plasma, serum, urine, saliva, cerebrospinal fluid, etc., may be obtained from the subject being screened. Alternatively, cells expressing the

protein on their surface, *e.g.*, suitable neuronal cells for the detection of LSAMP protein, may be obtained by simple, conventional means. If the protein is a receptor or other cell surface structure, it can be detected and quantified by well-known methods such as flow cytometry, immunofluorescence, immunocytochemistry or immunohistochemistry, or the like (see *supra*).

In a preferred embodiment, the detection or diagnosis is performed on a sample from a kidney tumor, *e.g.*, a tissue biopsy, a fresh-frozen sample, or a paraffin-embedded tissue section. Methods of preparing all of these sample types are conventional and well known in the art. Biopsy material and fresh-frozen samples can be extracted by conventional procedures to obtain proteins or polypeptides. In one embodiment, paraffin-embedded blocks are sectioned and analyzed directly without such extraction.

Another embodiment of the invention is a method for inhibiting the growth, transformation or other cancer-associated property of a tumor cell, preferably a CC-RCC cell, which is characterized by reduced expression of the *LSAMP* and/or *NORE1* genes compared to a normal kidney cell or a baseline value. The method comprises contacting the cell with an effective amount of an agent which stimulates the expression of the LSAMP and/or NORE1 polypeptide. By an "effective amount" is meant an amount that leads to a measurable reduction of such expression measured at the RNA or protein level.. Methods of contacting a cell are conventional and include injection or other forms of administration and my be done using liposomes, electroporation, microinjection or the like. The cell may be contacted *in vitro* or *in vivo*.

Another embodiment is a method for treating a subject suffering from cancer or a tumor, such as CC-RCC, in which at least some of the cells of the subject under-express the *LSAMP* and/or the *NORE1* gene compared to a baseline value. The method comprises

(1) administering to the subject an effective amount of LSAMP and/or NORE1 polypeptide or active fragment or variant thereof, or a nucleic acid encoding the polypeptide or active fragment or variant thereof which fragment or variant have the desired biological level of the LSAMP or NORE1 polypeptide; or

(2) administering an agent which stimulates, promotes or otherwise results in increased expression LSAMP or NORE1 polypeptide.

Methods of administering the polypeptide are conventional and include, *e.g.*, systemic administration or, preferentially, direct intratumoral administration. The subject may be any suitable animal, preferably a mammal, more preferably a human.

In the above nucleic acid embodiments, the polynucleotide being administered comprises sequences which encode the polypeptide (or variant or fragment), and which are operably linked to an expression control sequence such as a promoter. This polynucleotide may be cloned in a suitable vector, many examples of which are well known to those of skill in the art.

As used herein, the term "expression control sequence" means a polynucleotide sequence that regulates expression of a polypeptide encoded by a polynucleotide to which the control sequence is functionally ("operably") linked. Expression can be regulated at the level of the mRNA or polypeptide synthesis or stability. Thus, the "term expression control sequence" includes mRNA-related elements and protein-related elements, which include promoters, domains within promoters, upstream elements, enhancers, elements that confer tissue or cell specificity, response elements, ribosome binding sequences, transcriptional terminators, *etc.* An expression control sequence is operably linked to a nucleotide sequence (*e.g.*, a coding sequence) when the expression control sequence is positioned in such a manner to effect or achieve expression of the coding sequence. For example, when a promoter is operably linked 5' to a coding sequence, expression of the coding sequence is driven by the promoter. Suitable expression control sequences will be evident to the skilled worker.

Methods for generating polynucleotides and polypeptides for use in the methods, compositions and kits of the invention, are conventional. For example, polynucleotides can be isolated, *e.g.*, using sequence probes corresponding to the sequences indicated in the GenBank accession numbers provided elsewhere herein. The polynucleotides can be cloned into suitable vectors, and introduced into and replicated and/or expressed in suitable host cells. Procedures for carrying out these steps are conventional. Nucleic acids that have replicated in the cells, and polypeptides expressed in the cells, can be harvested and, if desired, purified, using conventional procedures. Some suitable molecular biology methods, for use in these and other aspects of the invention, are provided *e.g.*, in Sambrook, *et al.* (1989), *Molecular Cloning, a Laboratory Manual*, Cold Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Ausubel *et al.* (1995). *Current Protocols in Molecular Biology*, N.Y., John Wiley & Sons; Davis *et al.* (1986), *Basic Methods in Molecular Biology*, Elsevier Sciences Publishing,, Inc., New York; Hames *et al.* (1985), *Nucleic Acid Hybridization*, IL Press; Dracopoli *et al. Current Protocols in Human Genetics*, John Wiley & Sons, Inc.; and Coligan *et al. Current Protocols in Protein Science*, John Wiley & Sons, Inc.

Methods for providing a polynucleotide to a cell *in vitro, i.e.,* contacting the cell, are conventional and include, transfection, a gene gun, microinjection, electroporation, introduction by liposomes or with viral or non-viral vectors, *etc.*

For gene gun-mediated DNA injection, DNA-coated gold particles (*e.g.*, about 1 μg DNA/bullet) are delivered using a helium-driven gene gun (BioRad, Hercules, CA) with a discharge pressure of, for example, about 400 p.s.i. The Biojector 2000 (Bioject Inc., Portland, OR) is a needle-free jet injection device consisting of an injector and a disposable syringe. The orifice size controls the depth of penetration. For example, DNA (at between about 1 and 100 μg) may be delivered using the Biojector with a syringe nozzle. This may be done intradermally, intramuscularly or intratumorally. Follow-up injections using both methods can be repeated as needed, *e.g.*, at weekly intervals.

Methods of gene therapy or nucleic acid therapy, in which a polynucleotide of the invention is provided in a delivery vehicle, are well-known. The gene vehicle may be of viral or non-viral origin (see generally, Jolly, *Cancer Gene Therapy* 1:51-64 (1994) Kimura, *Human Gene Therapy* 5:845-852 (1994); Connelly, *Human Gene Therapy* 1:185-193 (1995); and Kaplitt, *Nature Genetics* 6:148-153 (1994). Vehicles for delivery of nucleic acid constructs including a coding sequence of a therapeutic embodiment of the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of the coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

Recombinant retroviruses constructed to carry or express a selected nucleic acid molecule of interest may be used. See, for example, EP 0415731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 93/11230; WO 93/10218; Vile *et al.*, , *Canc Res* 53:3860-3864 (1993); Vile *et al.*, *Canc. Res.* 53:962-967 (1993); Ram *et al.*, *Canc. Res.* 53:83-88 (1993); Takamiya *et al.*, *J. Neurosci. Res.* 33:493-503 (1992); Baba *et al.*, *J. Neurosurg.* 79:729-735 (1993); U.S. Patent No. 4,777,127; GB Patent No. 2,200,651; and EP 0345242. Preferred recombinant retroviruses include those described in WO 91/02805.

Packaging cell lines suitable for use with the above-described retroviral vector constructs may be readily prepared (WO 95/30763 and WO 92/05266), and used to create producer cell lines (also termed vector cell lines) for the production of recombinant vector particles. Preferred

embodiments of the invention utilize packaging cell lines made from human (such as HT1080 cells) or from mink parent cell lines, that result in production of recombinant retroviruses that survive inactivation in human serum.

Alphavirus-based vectors can function as gene delivery vehicles and be constructed from a wide variety of alphaviruses, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250 ATCC VR-1249; ATCC VR-532). Representative examples of such vector systems are described in U.S. Patents No. 5,091,309; 5,217,879; and 5,185,440; and PCT Publications WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994.

Delivery vehicles of the present invention can also employ parvovirus such as adeno-associated virus (AAV) vectors. See, for example, Srivastava, WO 93/09239, Samulski *et al.*, *J. Vir.* 63:3822-3828 (1989); Mendelson *et al.*, *Virol.* 166:154-165 (1988); and Flotte *et al.*, *P.N.A.S.* 90:10613-10617 (1993).

Representative examples of adenoviral vectors are described by Berkner, *Biotechniques* 6:616-627; Rosenfeld *et al.*, *Science* 252:431-434 (1991); WO 93/19191; Kolls *et al.*, *Proc. Natl. Acad Sci.USA. 91:*215-219 (1994); Kass-Eisler *et al.*, *Proc. Natl. Acad Sci.USA* 90:11498-11502 (1993); Guzman *et al.*, *Circulation* 88:2838-2848 (1993); Guzman *et al.*, *Cir. Res.* 73:1202-1207 (1993); Zabner *et al.*, *Cell* 75:207-216 (1993); Li *et al.*, *Hum. Gene Ther.* 4:403-409 (1993); Cailaud *et al.*, *Eur. J. Neurosci.* 5: 1287-1291 (1993); Vincent *et al.*, *Nat. Genet.* 5:130-134 (1993); Jaffe *et al.*, *Nat. Genet.* 1:372-378 (1992); and Levrero *et al.*, *Gene* 101:195-202 (1992). Exemplary adenoviral nucleic acid therapy vectors useful herein are described in WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655. Administration of DNA linked to killed adenovirus is described in Curiel, *Hum. Gene Ther.* 3:147-154 (1992).

Other delivery vehicles and methods may be employed, including polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example, Curiel (*supra*); ligand-linked DNA, for example, see Wu, *J. Biol. Chem.* 264:16985-16987 (1989); eukaryotic cell delivery vehicles (U.S.S.N. 08/240,030, filed May 9, 1994, and 08/404,796); deposition of photopolymerized hydrogel materials; hand-held gene transfer particle gun (U.S. Patent 5,149,655); ionizing radiation (U.S. Patent No. 5,206,152 and WO 92/11033; nucleic acid charge neutralization or fusion with cell membranes. Additional approaches are described in

Philip, *Mol. Cell Biol.* 14:2411-2418 (1994) and Woffendin, *Proc. Natl. Acad. Sci. USA* 91:1581-1585 (1994) (a mechanical delivery system).

Naked DNA may also be employed. See, for example, WO 90/11092 and U.S. Patent 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into thr cytoplasm.

Use of liposomes as DNA delivery vehicles are described in U.S. Patent 5,422,120, PCT Patent Pub. WO 95/13796, WO 94/23697 and WO 91/14445, and EP 0 524 968.

Effective dosages and routes of administration of polypeptides or polynucleotides of the invention are conventional. The exact amount (effective dose) of the agent will vary from subject to subject, depending on the species, age, weight and general or clinical condition of the subject, the severity or mechanism of any disorder being treated, the particular agent or vehicle used, the method and scheduling of administration, and the like. A therapeutically effective dose can be determined empirically, by conventional procedures known to those of skill in the art. See, *e.g.*, *The Pharmacological Basis of Therapeutics*, Goodman and Gilman, eds., Macmillan Publishing Co., New York. For example, an effective dose can be estimated initially either in cell culture assays or in suitable animal models. The animal model may also be used to determine the appropriate concentration ranges and routes of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutic dose can also be selected by analogy to dosages for comparable therapeutic agents. In general, effective doses include between about 10 ng to about 100 mg up to a total dose of about 5g, depending on the route of administration, number of repeat administrations and other factors as noted above.

A variety of routes of administration may be used, including oral, respiratory, intranasal, intrarectal, intravaginal, sublingual, transdermal, extracorporeal, topical, intravenous, subcutaneous, intramuscular, intramedullary, or intraperitoneal injection, other parenteral routes, or the like. One of skill in the art will recognize particular cells, tissues or organs into which therapeutic agents of the invention can be administered, as appropriate for particular indications.

Another embodiment of the invention is a pharmaceutical composition comprising (a) an LSAMP and/or NORE1 polypeptide, or an active fragment or variant thereof, or (b) a

polynucleotide encoding an LSAMP and/or NORE1 polypeptide, or encoding an active fragment or variant of the polypeptide, wherein the polynucleotide is operably linked to an expression control sequence; and a pharmaceutically acceptable carrier.

Another embodiment of the invention is a kit, suitable for carrying out any method of the invention. For example, the invention includes a kit for detecting the presence and/or amount of an LSAMP and/or a NORE1 polypeptide in a tumor or pre-cancerous sample, such as CC-RCC or normal kidney cells susceptible of transformation to become CC-RCC, wherein the cells are characterized by a reduced amount compared to a baseline value of one or both of these polypeptides. The kit comprises one or more reagents for detecting the polypeptide, preferably an antibody specific for the polypeptide, which is preferably detectably labeled, and, optionally, one or more reagents for testing the binding of the antibody to a sample polypeptide and/or that one that facilitates detection of antibody binding.

Another embodiment is a kit for detecting the presence and/or amount of a polynucleotide encoding LSAMP and/or NORE1 polypeptide in a tumor or pre-cancerous sample, such as a CC-RCC tumor cells or normal kidney cells susceptible of transformation to become CC-RCC. Such cells are characterized by under-expression of LSAMP and/or NORE1 compared to a baseline value which is indicative that the cells are susceptible of development into cancer cells, primarily CC-RCC cells. The kit comprises a nucleic acid probe specific for a LSAMP- or NORE1-encoding polynucleotide, and, optionally, one or more reagents that facilitate hybridization of the probe to the sample polynucleotide, and/or that facilitate detection of the hybridized polynucleotide.

Another embodiment is a kit useful for inhibiting or reducing a cancer-associated property of a cell, comprising an LSAMP and/or NORE1 polypeptide, or an active fragment or variant thereof, and, optionally, means for introducing the polypeptide into the cell and/or for measuring the cancer-associated property.

In another embodiment, the kit is suitable for treating a subject suffering from a cancer such as CC-RCC, the kit comprising an LSAMP and/or NORE1 polypeptide, or an active fragment or variant thereof, and, optionally, means for administering the polypeptide to the subject.

In other embodiments, the kit comprises, instead of the polypeptides, a nucleic acid encoding LSAMP and/or NORE1, or encoding active fragments or variants of the polypeptides, wherein the polynucleotides are operably linked to expression control sequences.

Optionally, kits of the invention comprise instructions for performing the method for which the kit is intended and/or for analyzing and/or evaluating the assay results as generated by the method. A kit may further comprise a support on which a cell can be propagated (*e.g.*, a tissue culture vessel) or a support to which a reagent used in the method is immobilized. Other optional elements of the present kit include suitable buffers, media components, or the like; reagents for performing suitable controls; a computer or computer-readable medium for storing and/or evaluating the assay results; containers; or packaging materials. The reagents of the kit may be in containers in which the reagents are stable, *e.g.*, in lyophilized form or stabilized liquids. The reagents may also be in single use form, *e.g.*, in single dosage form for use as therapeutics, or in single reaction form for diagnostic use.

Another embodiment of the invention is an antibody specific for an epitope of the LSAMP or he NORE1 polypeptide. Such antibodies are useful, not only for diagnostic procedures, as discussed above, but also for experimental purposes, *e.g.*, for elucidating the mechanisms of CC-RCC carcinogenesis. Some of the types of suitable antibodies, and methods for using them, were discussed above.

EXAMPLES

EXAMPLE I

Mapping of Breakpoints

The inventors have previously mapped the constitutional t(1;3)-associated breakpoints to bands 1q32.1 and 3q13.3 in a family with four cases of CC-RCC (Kanayama *et al.*, 2001). In this study, the inventors cloned the breakpoints of t(1;3)(q32.1;q13.3) by using a strategy that combined FISH, Southern blot, long-range PCR, and DNA sequencing. FISH experiments allowed narrowing of the breakpoint regions to a 20- to 30-kb range on both affected chromosomes (Figure 1A). These were further refined via Southern blot analyses and restriction mapping to approximately 2 kb (Figure 1B and 1C). Assisted by information from human genome sequence databases and BAC clone databases, several sets of specific primers were designed around the breakpoints and long-range PCR was performed to amplify the breakpoint fragments (Figure 2A). A 2.15 kb der(1)-breakpoint and a 3.25 kb der(3)-breakpoint were amplified and subcloned into TA-cloning vector (Invitrogen, USA) (Figure 2A). Subsequent DNA sequencing of the breakpoint fragments resulted in the identification of both breakpoints (Figure 2; also see Table 1).

Table 1.
Sequences and positions of the synthetic oligonucleotides used in the study.

Target	Direction	Sequence (5′-3′)	SEQ ID NO:
		For genomic PCR of the fusion genes	
NORE1-LSAMP	Forward	GGAGAAAGAGAGACCAGGACAAA (*NORE1*)	1
NORE1-LSAMP	Reverse	GCTTCCCAGGTTCAAGTGATTC (*LSAMP*)	2
NORE1-LSAMP	Sequencing Forward	AGCGATCATCCTGCCTTG (*NORE1*)	3
NORE1-LSAMP	Sequencing Forward	CATGCCAGGCCCATAAATAG (*NORE1*)	4
NORE1-LSAMP	Sequencing Forward	GTGGCCTGCAAAACCTAAC (*NORE1*)	5
LSAMP-NORE1A	Forward	AATCCAGGTCTCTCTGCTCCAA (*LSAMP*)	6
LSAMP-NORE1A	Reverse	CTCTGAGTGAGTCACGTGGCTT (*NORE1*)	7
LSAMP-NORE1A	Forward	CCTTCCTTTTCTCCATAGCACT (*LSAMP*)	8
LSAMP-NORE1A	Forward	ATTGTGGAAAATGGAGCTTC (*LSAMP*)	9
		For fusion transcript analyses	
NORE1A-LSAMP	Forward	AGTCAGCAGGAGGGTTTATCC (*NORE1A*)	10
NORE1A-LSAMP	Reverse	CCCTTCCAGTTGGTGTAAGGT (*LSAMP*)	11
LSAMP-NORE1A	Forward	TTCTCTGGAATACAGCCTCCG (*LSAMP*)	12
LSAMP-NORE1A	Reverse	GCGTGTTGTAGCTGTCGATC (*NORE1A*)	13
NORE1B-LSAMP	Forward	GCAGCATGAGCAGTGGGTAC (*NORE1B*)	14
NORE1B-LSAMP	Reverse	TTTGCCTGACTGCTCCCTG (*LSAMP*)	15
		For mutation analyses	
***NORE1*gene**			16
NORE1A Exon 1	Forward	TCCTTCCTGCCACTCCGACTC	17
NORE1A Exon 1	Reverse	TCCCAAGAACTCACAACAAAACC	18
NORE1A Exon 1	Sequencing Forward	TCCTTCCTGCCACTCCGACT	19
NORE1A Exon 1	Sequencing Reverse	TCCTCGCGCCTCTGTGTCCC	20
NORE1A Exon 2	Forward	TCCAAGGTTATTTCTCTGGGTG	21
NORE1A Exon 2	Reverse	GAGTTCTCTGTGTCACTTCCCC	22
NORE1A Exon 3	Forward	CTGGATGCTCACTTCTTGGTTAG	23
NORE1A Exon 3	Reverse	CAGAATTCAGAGTGAGGGCAG	24
NORE1A Exon 4	Forward	AGAACTCAAGGAGACAGGTGGG	25

Target	Direction	Sequence (5′-3′)	SEQ ID NO:
NORE1A Exon 4	Reverse	AGATCTGAACACCACATGGGC	26
NORE1A Exon 5	Forward	CACCTCTGCATTTCCAATCCTT	27
NORE1A Exon 5	Reverse	GTGGCTCCCACCTATGTGAG	28
NORE1A Exon 6	Forward	CAGGGTCTCTCAGGTCGTGTCA	29
NORE1A Exon 6	Reverse	CCCCCATGCAAACACTTGTC	30
NORE1B Exon1	Forward	CCCGCTGAAAGAAACGCAGG	31
NORE1B Exon1	Reverse	ATGCTCAGCCCTCAGGGCAA	32
***LSAMP*gene**			
LSAMP Exon1	Forward	AGTGGAAAGGACCATAAACTGGC	33
LSAMP Exon1	Reverse	TGGAGTTCAAGGAGATCAGACAC	34
LSAMP Exon2	Forward	ATGACATCCATCCACTGGATG	35
LSAMP Exon2	Reverse	TGCAACTCCCACCTCTTTCTTA	36
LSAMP Exon3	Forward	AGATGGCAAGCATGGGTCTTA	37
LSAMP Exon3	Reverse	TCAGCAGAATTCCAGGAGCA	38
LSAMP Exon4	Forward	CTGCTTCTGTGGAATCTGATGTC	39
LSAMP Exon4	Reverse	CAAAGACCAAGTCCTGCCCTT	40
LSAMP Exon5	Forward	CTCCCTTCCTGCCTCTCTCTAA	41
LSAMP Exon5	Reverse	GCTTAAGAGCTACAGGCCCC	42
LSAMP Exon6	Forward	TCCTTTTCCTCCAGTGTCAGG	43
LSAMP Exon6	Reverse	TGCTATGCACAGGAGTTGAGAA	44
LSAMP Exon7	Forward	CTTCTTGGGCTGCACATAAGTG	45
LSAMP Exon7	Reverse	ACGGTCTCCCCCATCTCTCT	46
***VHL* gene**			
VHL Exon1	Forward	TGTAAAACGACGGCCAGTCGAAGAGTACGGCCCTGAAGAAGAC	47
VHL Exon1	Reverse	CAGGAAACAGCTATGACCCAGTACCCTGGATGTGTCCTGCCTC	48
VHL Exon2	Forward	TGTAAAACGACGGCCAGTAGACGAGGTTTCACCACGTTAGC	49
VHL Exon2	Reverse	CAGGAAACAGCTATGACCGTCCTCTATCCTGTACTTACCAC	50
VHL Exon3	Forward	TGTAAAACGACGGCCAGTCTGAGACCCTAGTCTGCCACTGAGGAT	51
VHL Exon3	Reverse	CAGGAAACAGCTATGACCCAAAAGCTGAGATGAAACAGTGTAAGT	52

Target	Direction	Sequence (5′-3′)	SEQ ID NO:
		For genomic PCR of the fusion genes	
NORE1-LSAMP	Forward	GGAGAAAGAGAGACCAGGACAAA (*NORE1*)	53
NORE1-LSAMP	Reverse	GCTTCCCAGGTTCAAGTGATTC (*LSAMP*)	54
NORE1-LSAMP	Sequencing Forward	AGCGATCATCCTGCCTTG (*NORE1*)	55
NORE1-LSAMP	Sequencing Forward	CATGCCAGGCCCATAAATAG (*NORE1*)	56
NORE1-LSAMP	Sequencing Forward	GTGGCCTGCAAAACCTAAC (*NORE1*)	57
LSAMP-NORE1A	Forward	AATCCAGGTCTCTCTGCTCCAA (*LSAMP*)	58
LSAMP-NORE1A	Reverse	CTCTGAGTGAGTCACGTGGCTT (*NORE1*)	59
LSAMP-NORE1A	Forward	CCTTCCTTTTCTCCATAGCACT (*LSAMP*)	60
LSAMP-NORE1A	Forward	ATTGTGGAAAATGGAGCTTC (*LSAMP*)	61
		For fusion transcript analyses	
NORE1A-LSAMP	Forward	AGTCAGCAGGAGGGTTTATCC (*NORE1A*)	62
NORE1A-LSAMP	Reverse	CCCTTCCAGTTGGTGTAAGGT (*LSAMP*)	63
LSAMP-NORE1A	Forward	TTCTCTGGAATACAGCCTCCG (*LSAMP*)	64
LSAMP-NORE1A	Reverse	GCGTGTTGTAGCTGTCGATC (*NORE1A*)	65
NORE1B-LSAMP	Forward	GCAGCATGAGCAGTGGGTAC (*NORE1B*)	66
NORE1B-LSAMP	Reverse	TTTGCCTGACTGCTCCCTG (*LSAMP*)	67
		For mutation analyses	
***NORE1*gene**			
NORE1A Exon 1	Forward	TCCTTCCTGCCACTCCGACTC	69
NORE1A Exon 1	Reverse	TCCCAAGAACTCACAACAAAACC	71
NORE1A Exon 1	Sequencing Forward	TCCTTCCTGCCACTCCGACT	72
NORE1A Exon 1	Sequencing Reverse	TCCTCGCGCCTCTGTGTCCC	73
NORE1A Exon 2	Forward	TCCAAGGTTATTTCTCTGGGTG	74
NORE1A Exon 2	Reverse	GAGTTCTCTGTGTCACTTCCCC	75
NORE1A Exon 3	Forward	CTGGATGCTCACTTCTTGGTTAG	76
NORE1A Exon 3	Reverse	CAGAATTCAGAGTGAGGGCAG	77
NORE1A Exon 4	Forward	AGAACTCAAGGAGACAGGTGGG	78
NORE1A Exon 4	Reverse	AGATCTGAACACCACATGGGC	79

Target	Direction	Sequence (5′-3′)	SEQ ID NO:
NORE1A Exon 5	Forward	CACCTCTGCATTTCCAATCCTT	80
NORE1A Exon 5	Reverse	GTGGCTCCCACCTATGTGAG	81
NORE1A Exon 6	Forward	CAGGGTCTCTCAGGTCGTGTCA	82
NORE1A Exon 6	Reverse	CCCCCATGCAAACACTTGTC	83
NORE1B Exon1	Forward	CCCGCTGAAAGAAACGCAGG	84
NORE1B Exon1	Reverse	ATGCTCAGCCCTCAGGGCAA	85
***LSAMP*gene**			
LSAMP Exon1	Forward	AGTGGAAAGGACCATAAACTGGC	86
LSAMP Exon1	Reverse	TGGAGTTCAAGGAGATCAGACAC	87
LSAMP Exon2	Forward	ATGACATCCATCCACTGGATG	88
LSAMP Exon2	Reverse	TGCAACTCCCACCTCTTTCTTA	89
LSAMP Exon3	Forward	AGATGGCAAGCATGGGTCTTA	90
LSAMP Exon3	Reverse	TCAGCAGAATTCCAGGAGCA	91
LSAMP Exon4	Forward	CTGCTTCTGTGGAATCTGATGTC	92
LSAMP Exon4	Reverse	CAAAGACCAAGTCCTGCCCTT	93
LSAMP Exon5	Forward	CTCCCTTCCTGCCTCTCTCTAA	94
LSAMP Exon5	Reverse	GCTTAAGAGCTACAGGCCCC	95
LSAMP Exon6	Forward	TCCTTTTCCTCCAGTGTCAGG	96
LSAMP Exon6	Reverse	TGCTATGCACAGGAGTTGAGAA	97
LSAMP Exon7	Forward	CTTCTTGGGCTGCACATAAGTG	98
LSAMP Exon7	Reverse	ACGGTCTCCCCCATCTCTCT	99
***VHL* gene**			
VHL Exon1	Forward	TGTAAAACGACGGCCAGTCGAAGAGTACGGCCCTGAAGAAGAC	100
VHL Exon1	Reverse	CAGGAAACAGCTATGACCCAGTACCCTGGATGTGTCCTGCCTC	101
VHL Exon2	Forward	TGTAAAACGACGGCCAGTAGACGAGGTTTCACCACGTTAGC	102
VHL Exon2	Reverse	CAGGAAACAGCTATGACCGTCCTCTATCCTGTACTTACCAC	103
VHL Exon3	Forward	TGTAAAACGACGGCCAGTCTGAGACCCTAGTCTGCCACTGAGGAT	104
VHL Exon3	Reverse	CAGGAAACAGCTATGACCCAAAAGCTGAGATGAAACAGTGTAAGT	105

The cloning of the breakpoints led to the identification of two breakpoint-spanning genes, *NORE1* on 1q32.1 and *LSAMP* on 3q13.3 (Figure 2B). To investigate whether fusion proteins resulting from the chromosome translocation are involved in tumorigenesis of CC-RCC, Northern blot analysis and RT-PCR were carried out to detect any fusion transcript of *NORE1* and *LSAMP* (see Table A in supplemental data). No detectable fusion transcripts were found in the FRCC3 and FRCC5 cell lines from two patients in the t(1;3) family. The possible sequence combination from *NORE1* and *LSAMP* were also tested. Since *NORE1* lies in the positive DNA strand and *LSAMP* in the reverse strand, there is little likelihood for them to form any *NORE1-LSAMP* or *LSAMP-NORE1* fusion proteins.

Given the association between chromosome 3 translocations and CC-RCC susceptibility (van Kessel *et al.*, 1999; Bodmer *et al.*, 2002c), the gene *LSAMP* was investigated. *LSAMP* is composed of seven exons and is disrupted in intron 2 by the breakpoint (Figure 2B). To elucidate whether genetic changes in *LSAMP* play a role in CC-RCC, *LSAMP* mutation analysis was performed in 9 CC-RCC cell lines and in 53 sporadic and 4 familial tumors. No *LSAMP* mutation was detected. However, epigenetic silencing in association with hypermethylation, the most common form of inactivation for many tumor suppressor genes (Jones *et al.*, 2002), could still occur. First, RT-PCR analysis showed that LSAMP was down-regulated in all nine RCC cell lines (Figure 3A). Furthermore, the *LSAMP* promoter was methylated in 7/9 CC-RCC cell lines (78%), 14/53 sporadic CC-RCCs (26%), and all 4 familial CC-RCCs tumors from the t(1;3) family (Figure 3B). In association with the promoter-methylation status, LSAMP expression in ten examined tumors with *LSAMP*-promoter methylation was also down-regulated (Figure 3A). Of the *LSAMP*-promoter-methylated cell lines and tumors, all presented complete methylation except two cell lines and one sporadic tumor. Furthermore, in the four familial tumors (FT1 to FT4), one *LSAMP* allele was breakpoint-disrupted followed by the loss of the der(3) chromosome shown in our previous study (Kanayama *et al.*, 2001), and the other copy was hypermethylated (Figure 3B), implying *LSAMP* may undergo bi-allelic inactivation. These observations suggest that *LSAMP* is involved in CC-RCC, though further functional studies are needed to elucidate its mechanism.

The 1q32.1 breakpoint-disrupted gene, *NORE1*, also appeared to be an excellent candidate CC-RCC suppressor gene. *NORE1* undergoes alternative splicing, resulting in two isoforms, *NORE1A* and *NORE1B*. The breakpoint disrupted both *NORE1A* and *NORE1B* (Figure 2B). NORE1 is homologous to a family of RAS binding proteins, including RASSF1,

rat Maxp1, and murine Norel (Vavvas *et al.*, 1998; Dammann *et al.*, 2000; Vos *et al.*, 2000; Ortiz-Vega *et al.*, 2002; Tommasi *et al.*, 2002) that have been proposed to be effectors for the small GTPase. Maxp1, Norel and RASSF1 have been shown to induce apoptosis (Vos *et al.*, 2000; Khokhlatchev *et al.*, 2002). Other studies, however, have shown that Norel family members are cytostatic and modulate cyclinD1 levels, thereby influencing the activity of cell cycle-dependent kinases (Khokhlatchev *et al.*, 2002). *RASSF1* maps to 3p21, a region of frequent LOH in CC-RCC (van den Berg *et al.*, 1997; Dammann *et al.*, 2000), and this gene has recently been shown to be epigenetically inactivated in kidney cancer (Dreijerink *et al.*, 2001; Morrissey *et al.*, 2001; Yoon *et al.*, 2001). Thus, the inventors proceeded to investigate *NORE1* as a candidate RCC suppressor.

Mutation screening and methylation analysis were performed on *NORE1* in all the RCC cell lines and tumors. Two alterations, GTG(Val189)>ATG(Met189) and CGG(Arg248)>CAG(Gln248), were identified (see Figure 6). The former was present in 5% of the 100 tested normal subjects, whereas the latter was not found in any of them. As both were also present in the matched normal kidney tissues, it is likely that they represent polymorphisms. The inventors then perceived that *NORE1A* expression was also down-regulated in the 9 RCC cell lines, and the *NORE1A* promoter was methylated in 6/9 RCC cell lines and 17/53 (32%) sporadic RCC tumors (Figure 3A and 3C), whereas methylation in the *NORE1B* promoter was detected only in RCC cell lines A-498 and A-704. *NORE1A* expression in examined 10 of the 17 affected tumors was also down-regulated (Figure 3A). Two normal kidney control samples (N3 and N44) also showed *NORE1A* promoter methylation at lower extents compared with their matched tumors (3T and 44T), probably due to contamination from the tumor tissues. Interestingly, *NORE1A*-promoter methylation does not overlap with *LSAMP*-promoter methylation except in four tumors. These results suggest that *NORE1A* is also associated with sporadic CC-RCC. Yet, unlike the methylation situation in *LSAMP*, only 1/4 hereditary tumors showed even slight *NORE1A* promoter methylation, indicating one wild-type allele of *NORE1A* still exists in these hereditary tumors. Whether *NORE1A* undergoes haploinsufficiency in tumorigenesis remains undetermined.

In addition, 7/14 tumors (50%) with *LSAMP*-promoter methylation showed loss of heterozygosity (LOH) of the *LSAMP* locus. However, LOH was also observed in 17/39 tumors (44%) without *LSAMP*-promoter methylation. Similar LOH results were obtained on *NORE1A*

(methylated, 5/17 [29%]; unmethylated, 8/36, [22%]), indicating that the LOH may be correlated with CC-RCC tumorigenesis, but is not methylation-dependent.

Promoter methylation in both LSAMP and NORE1A may also be linked to other types of cancers. *NORE1A*-promoter methylation has recently been detected in cancer cell lines and in 24% NSCLC (Hesson *et al.*, 2003). Here the inventors found that the *LSAMP* promoter was methylated in 5/19 (26%) colorectal cancers.

The exact role of these genes in tumorigenesis is unclear. Without wishing to be bound to any particular mechanism, potential roles for these genes are discussed below. In the familial cases, the underlying mechanism appears to be the three-step model of chromosome 3 translocation-related hereditary CC-RCC tumorigenesis (Bodmer *et al.*, 1998; Kanayama *et al.*, 2001; Bodmer *et al.*, 2002c). Considering the complexity of the multistep process in tumorigenesis, the possibility exists that the breakpoint-disrupted genes, especially *LSAMP*, may contribute to the occurrence of familial tumors by acting as components in the three-step model of tumorigenesis of hereditary CC-RCC. The inventors have previously demonstrated that four examined familial CC-RCC tumors lost the der(3) chromosome and two of them carry *VHL* mutations, supporting the three-step model of tumorigenesis (Kanayama *et al.*, 2001). Here, the inventors supplement this model with our *LSAMP* and *NORE1A* data. The constitutional translocation t(1q;3q) and disruption of a copy each of *LSAMP* and *NORE1*, as the first set of steps of tumorigenesis, act as the predisposing factors in development of CC-RCC. The translocation also results in the increased susceptibility to somatic loss of the chromosome der(3). The following non-disjunctional loss of der(3) deletes a copy each of the RCC-related genes in chromosome 3 (e.g., *VHL*, *RASSF1A*), which further increases the predisposition to CC-RCC. This second set of steps will accelerate the transformation process and cellular growth, leading to the third set of steps involving either the inactivation of the other *VHL* allele (e.g. somatic mutation) or the genetic/epigenetic alternations in other CC-RCC-related genes including *LSAMP* in the remaining copy of chromosome 3. These factors may act synergistically and finally lead to the occurrence of CC-RCC.

Epigenetic inactivation of these genes can be reversed by demethylation treatment with the DNA methylation inhibitor 5-aza-2'-deoxycytidine (5-aza-CdR). The demethylation treatment resulted in significantly increased expression of LSAMP and NORE1 in eight cell lines (Figure 3A), indicating that repression is at least in part mediated by methylation.

Finally, to further evaluate the role of *LSAMP* and *NORE1* as tumor suppressor candidates in cancer, enhanced green fluorescent protein *EGFP-LSAMP*, *-NORE1A*, and *-Nore1* expression plasmids were microinjected or transfected into two RCC cell lines, A-498 and/or Caki-1, in which the *LSAMP* and *NORE1A* promoters were methylated, cells were then counted at indicated times, and were monitored for cell number and/or proliferation. Alternatively, cells were monitored by epi-fluorescence/phase-contrast microscopy to evaluate proliferation, fluorescent protein expression, or apoptosis. While cells expressing EGFP continued to proliferate at rates similar to those of uninjected neighbors, cells expressing EGFP-LSAMP, -NORE1A and -Nore1 failed to divide (Figure 4B). There was no evidence of apoptosis in any of the experiments. This growth inhibition role was also demonstrated in 293-T cells stably transformed with an inducible *Nore1* gene by lipid-mediated transfection (see Figure 7C).

The inventors also observed that EGFP-LSAMP seemed to be cytoplasmic, and EGFP-NORE1A appeared in both cytosol and nucleus. EGFP-Nore1 was predominantly nuclear and tended to occupy discrete puncta within the nucleus (Figure 4A). This was observed in both formaldehyde-fixed and living cells; thus, the localization was unlikely to be due to a fixation artifact. Furthermore, the nuclear localization of EGFP-Nore1 was also confirmed in the transfected Caki-1 RCC cell line and in the 293-T cells by nuclear fractionation (see Figures 7Aa and 7B).

These observations are consistent with a growth suppression role for LSAMP, NORE1A, and NORE1. Also, despite the presence of a putative Ras-association region, the results suggest that this nuclear Nore1 protein may not be a *bona fide* Ras effector, whose family members tend to be lipid-modified, membrane-bound, positive regulators of cell proliferation. Further investigation into its role in growth regulation (potentially through the regulation of cyclin D1 and G1/S progression) and its role in the nucleus are desirable.

Based on these data, LSAMP, and NORE1A (a homolog of 3p21-tumor suppressor RASSF1A), represent new tumor suppressor candidates, and presumably act as components in the multistep process of CC-RCC tumorigenesis. Inactivation or reduced expression of both LSAMP and NORE1A also appears to be involved in the occurrence of other types of tumors. Further studies of these genes may lead to the elucidation of novel mechanisms of tumorigenesis.

GenBank accession numbers and Sequences of NORE1A, LSAMP, etc.

NORE1A: **GenBank Accession No. NM_031437**

Nucleic Acid: SEQ ID NO: 120 (coding: 64-1236); Amino acid: SEQ ID NO:121,

```
   1 cgggagtagc gcagtcgcca aagccgccgc tgccaaagct gccgccacta gccgggcatg
  61 gccatggcgt ccccggccat cgggcagcgc ccgtacccgc tactcttgga ccccgagccg
 121 ccgcgctatc tacagagcct gagcggcccc gagctaccgc cgccgccccc cgaccggtcc
 181 tcgcgcctct gtgtcccggc gcccctctcc actgcgcccg gggcgcgcga ggggcgcagc
 241 gcccggaggg ctgcccgggg gaacctggag cccccgcccc gggcctcccg acccgctcgc
 301 ccgctccggc ctggtctgca gcagagactg cggcggcggc ctggagcgcc ccgaccccgc
 361 gacgtgcgga gcatcttcga gcagccgcag gatcccagag tcccggcgga gcgaggcgag
 421 gggcactgct tcgccgagtt ggtgctgccg ggcggccccg gctggtgtga cctgtgcgga
 481 cgagaggtgc tgcggcaggc gctgcgctgc actaactgta aattcacctg tcacccagaa
 541 tgccgcagcc tgatccagtt ggactgcagt cagcaggagg gtttatcccg ggacagaccc
 601 tctccagaaa gcaccctcac cgtgaccttc agccagaatg tctgtaaacc tgtggaggag
 661 acacagcgcc cgcccacact gcaggagatc aagcagaaga tcgacagcta caacacgcga
 721 gagaagaact gcctgggcat gaaactgagt gaagacggca cctacacggg tttcatcaaa
 781 gtgcatctga aactccggcg gcctgtgacg gtgcctgctg ggatccggcc ccagtccatc
 841 tatgatgcca tcaaggaggt gaacctggcg gctaccacgg acaagcggac atccttctac
 901 ctgcccctag atgccatcaa gcagctgcac atcagcagca ccaccaccgt cagtgaggtc
 961 atccaggggc tgctcaagaa gttcatggtt gtggacaatc cccagaagtt tgcacttttt
1021 aagcggatac acaaggacgg acaagtgctc ttccagaaac tctccattgc tgaccgcccc
1081 ctctacctgc gcctgcttgc tgggcctgac acggaggtcc tcagctttgt gctaaaggag
1141 aatgaaactg gagaggtaga gtgggatgcc ttctccatcc ctgaacttca gaacttcctc
1201 tcctcctggt gcattcagat ttatttgtat tattaattat tattttgcaa cagacacttt
1261 ttctcaggac atctctggca ggtgcatttg tgcctgccca gcagttccag ctgtggcaaa
1321 agtctcttcc atggacaagt gtttgcacga gggttcagct gtgcccgccc ccaggctgtg
1381 ccccaccaca gattctgcca aggatcagaa ctcatgtgaa acaaaacagct gacgtcctct
1441 ctcgatctgc aagcctttca ccaaccaaat agttgcctct ctcgtcacca aactggaacc
1501 tcacaccagc cggcaaagga aggaagaaag gttttagagc tgtgtgttct ttctctggca
1561 ttgattcctc tttgagttct cttacttgcc acgtacagga ccattattta tgagtgaaaa
1621 gttgtagcac attccttttg caggtctgag ctaagcccct gaaagcaggg taatgctcat
1681 aaaaggactg ttcccgcggc cccaaggtgc ctgttgttca cacttaaggg aagtttataa
1741 agctactggc cccagatgct cagggtaagg agcaccaaag ctgaggctgg ctcagagatc
1801 tccagagaag ctgcagcctg ccctggccct ggctctggcc ctggcccaca ttgcacatgg
1861 aaacccaaag gcatatatct gcgtatgtgt ggtacttagt cacatctttg tcaacaaact
1921 gttcgttttt aagttacaaa tttgaattta atgttgtcat catcgtcatg tgtttcccca
1981 aagggaagcc agtcattgac catttaaaaa gtctcctgct aagtatggaa atcagacagt
2041 aagagaaagc caaaaagcaa tgcagagaaa ggtgtccaag ctgtcttcag ccttccccag
2101 ctaaagagca gaggagggcc tgggctactt gggttcccca tcggcctcca gcactgcctc
2161 cctcctccca ctgcgactct gggatctcca ggtgctgccc aaggagttgc cttgattaca
2221 gagaggggag cctccaattc ggccaacttg gagtcctttc tgttttgaag catgggccag
2281 acccggcact gcgctcggag agccggtggg cctggcctcc ccgtcgacct cagtgccttt
2341 ttgttttcag agagaaatag gagtagggcg agtttgcctg aagctctgct gctggcttct
2401 cctgccagga agtgaacaat ggcggcggtg tgggagacaa ggccaggaga gcccgcgttc
2461 agtatgggtt gagggtcaca gacctccctc ccatctgggt gcctgagttt tgactccaat
2521 cagtgatacc agaccacatt gacagggagg atcaaattcc tgacttacat ttgcactggc
2581 ttcttgttta ggctgaatcc taaaataaat tagtcaaaaa attccaacaa gtagccagga
2641 ctgcagagac actccagtgc agagggagaa ggacttgtaa ttttcaaagc agggctggtt
2701 ttccaaccca gcctctgaga aaccatttct ttgctatcct ctgccttccc aagtccctct
2761 tgggtcggtt caagcccaag cttgttcgtg tagcttcaga agttccctct ctgacccagg
2821 ctgagtccat actgcccctg atcccagaag gaatgctgac ccctcgtcgt atgaactgtg
2881 catagtctcc agagcttcaa aggcaacaca agctcgcaac tctaagattt ttttaaacca
2941 caaaaaccct ggttagccat ctcatgctca gccttatcac ttccctccct ttagaaactc
3001 tctccctgct gtatattaaa gggagcaggt ggagagtcat tttccttcgt cctgcatgtc
3061 tctaacatta atagaaggca tggctcctgc tgcaaccgct gtgaatgctg ctgagaacct
3121 ccctctatgg ggatggctat tttatttttg agaaggaaaa aaaaagtcat gtatatatac
```

```
3181 acataaaggc atatagctat atataaagag ataagggtgt ttatgaaatg agaaaattat
3241 tggacaattc agactttact aaagcacagt tagacccaag gcctatgctg aggtctaaac
3301 ctctgaaaaa agtatagtat cgagtacccg ttccctccca gaggtgggag taactgctgg
3361 tagtgccttc tttggttgtg ttgctcagtg tgtaagtgtt tgtttccagg atattttctt
3421 tttaaatgtc tttcttatat gggttttaaa aaaaagtaat aaaagcctgt tgcaaaaatg
3481 aaaaaaaaaa aaaaaaaa\

MASPAIGQRP YPLLLDPEPP RYLQSLSGPE LPPPPPDRSS RLCVPAPLST APGAREGRSA
RRAARGNLEP PPRASRPARP LRPGLQQRLR RRPGAPRPRD VRSIFEQPQD PRVPAERGEG
HCFAELVLPG GPGWCDLCGR EVLRQALRCT NCKFTCHPEC RSLIQLDCSQ QEGLSRDRPS
PESTLTVTFS QNVCKPVEET QRPPTLQEIK QKIDSYNTRE KNCLGMKLSE DGTYTGFIKV
HLKLRRPVTV PAGIRPQSIY DAIKEVNLAA TTDKRTSFYL PLDAIKQLHI SSTTTVSEVI
QGLLKKFMVV DNPQKFALFK RIHKDGQVLF QKLSIADRPL YLRLLAGPDT EVLSFVLKEN
ETGEVEWDAF SIPELQNFLS SWCIQIYLYY
```

NORE1B: **GenBank Accession No. AF445801**

Nucleic acid - SEQ ID NO: 122 (coding: 411-1208); Amino acid: SEQ ID NO:123

```
   1 gaactgcttt acgcgagggg caaggaaagg cgcgggaggc gggggaggtg cggagatggc
  61 gctctgcacg gcggcggagg gagggcgctg gcgccgggga cacgaaaccg cagagcccgg
 121 acgagtcagg gagtgaggcg cgagccgggc gcccggggct ctgcaggcgc aggcggcgcg
 181 gggacaggag caggttaccg ggccgcccga gcgctcgcac cccgctgaaa aaaacgcagg
 241 cggcccgccg gctttgcctg gtccgatacc cgaccagctc ccggctcggg gctcagagct
 301 aggggcttac gccaagcgga gcccggggag gggtgcccac ctccctccgc cgcatcccaa
 361 gcccggcccc cttgatgcgc tggcggcctc ggccgggaac tccggggtag atgaccgtgg
 421 acagcagcat gagcagtggg tactgcagcc tggacgagga actggaagac tgcttcttca
 481 ctgctaagac taccttttc agaaatgcgc agagcaaaca tctttcaaag aatgtctgta
 541 aacctgtgga ggagacacag cgcccgccca cactgcagga gatcaagcag aagatcgaca
 601 gctacaacac gcgagagaag aactgcctgg gcatgaaact gagtgaagac ggcacctaca
 661 cgggtttcat caaagtgcat ctgaaactcc ggcggcctgt gacggtgcct gctgggatcc
 721 ggccccagtc catctatgat gccatcaagg aggtgaacct ggcggctacc acggacaagc
 781 ggacatcctt ctacctgccc ctagatgcca tcaagcagct gcacatcagc agcaccacca
 841 ccgtcagtga ggtcatccag gggctgctca agaagttcat ggttgtggac aatccccaga
 901 agtttgcact ttttaagcgg atacacaagg acggacaagt gctcttccag aaactctcca
 961 ttgctgaccg ccccctctac ctgcgcctgc ttgctgggcc tgacacggag gtcctcagct
1021 ttgtgctaaa ggagaatgaa actggagagg tagagtggga tgccttctcc atccctgaac
1081 ttcagaactt cctaacaatc ctggaaaaag aggagcagga caaaatccaa caagtgcaaa
1141 agaagtatga caagtttagg cagaaactgg aggaggcctt aagagaatcc cagggcaaac
1201 ctgggtaacc agtcctgctt cctctcctcc tggtgcattc agatttattt gtattattaa
1261 ttattatttt gcaacagaca ctttttctca ggacatctct ggcaggtgca tttgtgcctg
1321 cccagcagtt ccagctgtgg caaaagtctc ttccatggac aagtgtttgc acgggggttc
1381 agctgtgccc gcccccaggc tgtgccccac cacagattct gccaaggatc agaactcatg
1441 tgaaacaaac agctgacgtc ctctctcgat ctgcaagcct ttcaccaacc aaatagttgc
1501 ctctctcgtc accaaactgg aacctcacac cagccggcaa aggaaggaag aaaggtttta
1561 gagctgtgtg ttctttctct ggctttgatt cttctttgag ttctcttact tgccacgtac
1621 aggaccatta tttatgagtg aaaagttgta gcacattcct tttgcaggtc tgagctaaac
1681 ccctgaaagc agggtaatgc tcataaaagg actgttcccg cggccccaag gtgcctgttg
1741 ttcacactta agggaagttt ataaagctac tggccccaga tgctcagggt aaggagcacc
1801 aaagctgagg ctggctcaga gatctccaga gaagctgcag cctgccctgg ccctggctct
1861 ggccctggcc cacattgcac atggaaaccc aaaggcatat atctgcgtat gtgtggtact
1921 tagtcacatc tttgtcaaca aactgttcgt ttttaagtta caaatttgaa tttaatgttg
1981 tcatcatcgt catgtgtttc cccaaaggga agccagtcat tgaccattta aaaagtctcc
2041 tgctaagtat ggaaatcaga cagtaagaga aagccaaaaa gcaatgcaga gaaaggtgtc
2101 caagctgtct tcagccttcc ccagctaaag agcagaggag ggcctgggct acttgggttc
2161 cccatcggcc tccagcactg cctccctcct cccactgcga ctctgggatc tccaggtgct
2221 gcccaaggag ttgccttgat tacagagagg ggagcctcca attcggccaa cttggagtcc
2281 tttctgtttt gaagcatggg ccagacccgg cactgcgctc ggagagccgg tgggcctggc
```

```
2341 ctccccgtcg acctcagtgc ctttttgttt tcagagagaa ataggagtag ggcgagtttg
2401 cctgaagctc tgctgctggc ttctcctgcc aggaagtgaa caatggcggc ggtgtgggag
2461 acaaggccag gagagcccgc gttcagtatg ggttgagggt cacagacctc cctcccatct
2521 gggtgcctga gttttgactc caatcagtga taccagacca cattgacagg gaggatcaaa
2581 ttcctgactt acatttgcac tggcttcttg tttaggctga atcctaaaat aaattagtca
2641 aaaaattcca acaagtagcc aggactgcag agacactcca gtgcagaggg agaaggactt
2701 gtaattttca aagcagggct ggttttccaa cccagcctct gagaaaccat ttctttgcta
2761 tcctctgcct tcccaagtcc ctcttgggtc ggttcaagcc caagcttgtt cgtgtagctt
2821 cagaagttcc ctctccgacc caggctgagt ccatactgcc cctgatccca gaaggaatgc
2881 tgacccctcg tcgtatgaac tgtgcatagt ctccagagct tcaaaggcaa cacaagctcg
2941 caactctaag attttttaa accacaaaaa ccctggttag ccatctcatg ctcagcctta
3001 tcacttccct ccctttaga aaactctctc cctgctgtat attaaaggga gcaggtggag
3061 agtcattttc cttcgtcctg catgtctcta acattaatag aaggcatggc tcctgctgca
3121 accgctgtga atgctgctga gaacctccct ctatggggat ggctatttta tttttgagaa
3181 ggaaaaaaaa agtcatgtat atatacacat aaaggcatat agctatatat aaagagataa
3241 gggtgtttat gaaatgagaa aattattgga caattcagac tttactaaag cacagttaga
3301 cccaaggcct atgctgaggt ctaaacctct gaaaaaagta tagtatcgag tacccgttcc
3361 ctcccagagg tgggagtaac tgctggtagt gccttctttg gttgtgttgc tcagtgtgta
3421 agtgtttgtt tccaggatat tttcttttta aatgtctttc ttatatgggt tttaaaaaaa
3481 agtaataaaa gcctgttgc
```

```
MTVDSSMSSG YCSLDEELED CFFTAKTTFF RNAQSKHLSK NVCKPVEETQ RPPTLQEIKQ
KIDSYNTREK NCLGMKLSED GTYTGFIKVH LKLRRPVTVP AGIRPQSIYD AIKEVNLAAT
TDKRTSFYLP LDAIKQLHIS STTTVSEVIQ GLLKKFMVVD NPQKFALFKR IHKDGQVLFQ
KLSIADRPLY LRLLAGPDTE VLSFVLKENE TGEVEWDAFS IPELQNFLTI LEKEEQDKIQ
QVQKKYDKFR QKLEEALRES QGKPG
```

LSAMP NM_002338 cDNA

Nucleic acid: SEQ ID NO: 124 (coding: 501-1517); Amino acid SEQ ID NO:125

```
   1 ggggagagag gctctgggtt gctgctgctt ctgctgctgc tgctgctgtg tggctgtttc
  61 tgtacactca ctggcaggct tggtgccggc tccctcgccc gcccgcccgc cagcctggga
 121 aagtgggtta cagagcgaag gagctcagct cagacactgg cagaggagca tccagtcaca
 181 gagagaccaa acaagaaccc tttcctttgg cttcctcttc agctcttcca gagggcttgc
 241 tatttgcact ctctcttttg aaattgtgtt gcttttactt ttcacccttc tgcttgggtt
 301 ttatgagggc tttgttaagt cttagaggga aaagagactg agcgagggaa agagagaggc
 361 aaagtggaaa ggaccataaa ctggcaaagc ccgctctgcg ctcgctgtgg atgaaagccc
 421 cgtgttggtg aagcctctcc tcgcgagcag cgcgcacccc tccagagcac cccgcggacc
 481 cgcacctcgg cgtggccacc atggtcagga gagttcagcc ggatcggaaa cagttgccac
 541 tggtcctact gagattgctc tgccttcttc ccacaggact gcctgttcgc agcgtggatt
 601 ttaaccgagg cacggacaac atcaccgtga ggcaggggga cacagccatc ctcaggtgcg
 661 ttgtagaaga caagaactca aaggtggcct ggttgaaccg ttctggcatc atttttgctg
 721 gacatgacaa gtggtctctg gacccacggg ttgagctgga gaaacgccat tctctggaat
 781 acagcctccg aatccagaag gtggatgtct atgatgaggg ttcctacact tgctcagttc
 841 agacacagca tgagcccaag acctcccaag tttacttgat cgtacaagtc ccaccaaaga
 901 tctccaatat ctcctcggat gtcactgtga atgagggcag caacgtgact ctggtctgca
 961 tggccaatgg ccgtcctgaa cctgttatca cctggagaca ccttacacca actggaaggg
1021 aatttgaagg agaagaagaa tatctggaga tccttggcat caccagggag cagtcaggca
1081 aatatgagtg caaagctgcc aacgaggtct cctcggcgga tgtcaaacaa gtcaaggtca
1141 ctgtgaacta tcctcccact atcacagaat ccaagagcaa tgaagccacc acaggacgac
1201 aagcttcact caaatgtgag gcctcggcag tgcctgcacc tgactttgag tggtaccggg
1261 atgacactag gataaatagt gccaatggcc ttgagattaa gagcacggag ggccagtctt
1321 ccctgacggt gaccaacgtc actgaggagc actacggcaa ctacacctgt gtggctgcca
1381 acaagctggg ggtcaccaat gccagcctag tccttttcag acctgggtcg gtgagaggaa
1441 taaatggatc catcagtctg gccgtaccac tgtggctgct ggcagcatct ctgctctgcc
1501 ttctcagcaa atgttaatag aataaaaatt taaaaataaa aaaaaaaaaa aaaaaaaaaa
1561 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
```

```
1621 aaaaaaaaaa aaaaaaaaaa
```

```
MVRRVQPDRK QLPLVLLRLL CLLPTGLPVR SVDFNRGTDN ITVRQGDTAI LRCVVEDKNS
KVAWLNRSGI IFAGHDKWSL DPRVELEKRH SLEYSLRIQK VDVYDEGSYT CSVQTQHEPK
TSQVYLIVQV PPKISNISSD VTVNEGSNVT LVCMANGRPE PVITWRHLTP TGREFEGEEE
YLEILGITRE QSGKYECKAA NEVSSADVKQ VKVTVNYPPT ITESKSNEAT TGRQASLKCE
ASAVPAPDFE WYRDDTRINS ANGLEIKSTE GQSSLTVTNV TEEHYGNYTC VAANKLGVTN
ASLVLFRPGS VRGINGSISL AVPLWLLAAS LLCL
```

ASSF1A, GenBank Accession No. AF132675;

Nucleic acid: SEQ ID NO: 126 (coding: 39-1061); Amino acid SEQ ID NO:127

```
   1 agcgcccaaa gccagcgaag cacgggccca accgggccat gtcgggggag cctgagctca
  61 ttgagctgcg ggagctggca cccgctgggc gcgctgggaa gggccgcacc cggctggagc
 121 gtgccaacgc gctgcgcatc gcgcggggca ccgcgtgcaa ccccacacgg cagctggtcc
 181 ctggccgtgg ccaccgcttc cagcccgcgg ggcccgccac gcacacgtgg tgcgacctct
 241 gtggcgactt catctggggc gtcgtgcgca aaggcctgca gtgcgcgcat tgcaagttca
 301 cctgccacta ccgctgccgc gcgctcgtct gcctggactg ttgcgggccc cgggacctgg
 361 gctgggaacc cgcggtggag cgggacacga acgtggacga gcctgtggag tgggagacac
 421 ctgacctttc tcaagctgag attgagcaga agatcaagga gtacaatgcc cagatcaaca
 481 gcaacctctt catgagcttg aacaaggacg gttcttacac aggcttcatc aaggttcagc
 541 tgaagctggt gcgccctgtc tctgtgccct ccagcaagaa gccaccctcc ttgcaggatg
 601 cccggcgggg cccaggacgg ggcacaagtg tcaggcgccg cacttccttt tacctgccca
 661 aggatgctgt caagcaccta catgtgctgt cacgcacaag ggcacgtgaa gtcattgagg
 721 ccctgctgcg aaagttcttg gtggtagatg acccccgcaa gtttgcactc tttgagcgcg
 781 ctgagcgtca cggccaagtg tacttgcgga agctgttgga tgatgagcag cccctgcggc
 841 tgcggctcct ggcagggccc agtgacaagg ccctgagctt tgtcctgaag gaaaatgact
 901 ctggggaggt gaactgggac gccttcagca tgcctgaact acataacttc ctacgtatcc
 961 tgcagcggga ggaggaggag cacctccgcc agatcctgca gaagtactcc tattgccgcc
1021 agaagatcca agaggccctg cacgcctgcc cccttgggtg acctcttgta cccccaggtg
1081 gaaggcagac agcaggcagc gccaagtgcg tgccgtgtga gtgtgacagg gccagtgggg
1141 cctgtggaat gagtgtgcat ggaggccctc ctgtgctggg ggaatgagcc cagagaacag
1201 cgaagtagct tgctccctgt gtccacctgt gggtgtagcc aggtatggct ctgcacccct
1261 ctgccctcat tactgggcct tagtgggcca gggctgccct gagaagctgc tccaggcctg
1321 cagcaggagt ggtgcagaca gaagtctcct caatttttgt ctcagaagtg aaaatcttgg
1381 agaccctgca aacagaacag ggtcatgttt gcaggggtga cggccctcat ctatgaggaa
1441 aggttttgga tcttgaatgt ggtctcagga tatccttatc agagctaagg gtgggtgctc
1501 agaataaggc aggcattgag gaagagtctt ggtttctctc tacagtgcca actcctcaca
1561 caccctgagg tcagggagtg ctggctcaca gtacagcatg tgccttaatg cttcatatga
1621 ggaggatgtc cctgggccag ggtctgtgtg aatgtgggca ctggcccagg ttcatacctt
1681 atttgctaat caaagccagg gtctctccct caggtgtttt ttatgaagtg cgtgaatgta
1741 tgtaatgtgt ggtggcctca gctgaatgcc tcctgtgggg aaaggggttg gggtgacagt
1801 catcatcagg gcctggggcc tgagagaatt ggctcaataa agatttcaag atccaaaaaa
1861 aaaaaaaaaa aaa
```

```
MSGEPELIEL RELAPAGRAG KGRTRLERAN ALRIARGTAC NPTRQLVPGR GHRFQPAGPA
THTWCDLCGD FIWGVVRKGL QCAHCKFTCH YRCRALVCLD CCGPRDLGWE PAVERDTNVD
EPVEWETPDL SQAEIEQKIK EYNAQINSNL FMSLNKDGSY TGFIKVQLKL VRPVSVPSSK
KPPSLQDARR GPGRGTSVRR RTSFYLPKDA VKHLHVLSRT RAREVIEALL RKFLVVDDPR
KFALFERAER HGQVYLRKLL DDEQPLRLRL LAGPSDKALS FVLKENDSGE VNWDAFSMPE
LHNFLRILQR EEEEHLRQIL QKYSYCRQKI QEALHACPLG
```

Norel, AF053959;

Nucleic acid: SEQ ID NO: 128 (coding: 31-1272); Amin0 Acid: SEQ ID NO: 129

```
   1 gtagctgcgc cgctgactga ggccttggcc atggcttccc cggccatcgg gcaacgtccc
  61 tacccgctgc tcctagatcc cgagccgccg cggtatctgc agagtctggg tggcaccgag
 121 ccgccacctc ccgcccggcc gcgccgctgc atccccacgg ccctgatccc cgcggccggg
 181 gcgtcagagg atcgcggtgg caggaggagt ggccggaggg accccgaacc cacgccccga
 241 gactgccgac acgctcgccc tgtccggccc ggtctgcagc cgagactgcg gctgcgacct
 301 gggtcacacc gaccccgcga cgtgaggagc atcttcgagc agccgcagga tccccgcgtc
 361 ttggccgaga gaggcgaggg gcaccgtttc gtggaactgg cgctgcgggg cggtccgggc
 421 tggtgtgacc tgtgcggacg agaggtgctg cggcaggcgc tgcgctgcgc taattgtaaa
 481 ttcacctgcc actcggagtg ccgcagcctg atccagttgg actgcagaca gaaggggggc
 541 cctgccctgg atagacgctc tccaggaagc accctcaccc caaccttgaa ccagaatgtc
 601 tgtaaggcag tggaggagac acagcacccg cccacgatac aggagatcaa gcagaagatt
 661 gacagctata acagcaggga gaagcactgc ctgggcatga agctgagtga agatggcacc
 721 tacacaggtt tcatcaaagt gcatttgaag ctccgacggc cagtgacggt gcccgctgga
 781 tccggcccca gtccatctat ggatgccatt aaggaagtga accctgcagc caccacagac
 841 aagcggactt ccttctacct gccactcgat gccatcaagc agctacatat cagcagcacc
 901 accacggtta gtgaggtcat ccaggggctg ctcaagaagt tcatggttgt ggacaaccca
 961 cagaagtttg cacttttaa gcggatacac aaagatggac aagtgctctt ccagaaactc
1021 tccattgctg actatcctct ctaccttcgt ctgctcgctg ggcctgacac cgatgttctc
1081 agctttgtgc taaaggagaa tgaaactgga gaggtggagt gggatgcctt ttccattcct
1141 gaactccaga acttttaac tatcctggaa aaagaggagc aggacaagat ccatcaactg
1201 caaaagaagt acaacaaatt ccgtcagaaa ctggaagagg cattacgaga gtcccaaggg
1261 aagccggggt aaccagccga cttcctgtcc tctcagtgcc ctccaattta ttttattgtt
1321 aattattttg caacaaagag ttactgttaa gacacctctg gtggttccac cagtcgcctg
1381 cccagcagtt aacagatgtg gcacaaagtc tcttccacgc agtgtctatg cagggttccg
1441 attcctgcta acccaccaca ccatggctct ggagagcttc ccgcctggga tcagaactcc
1501 tgtggaatga ccagtgtttc cctgctcagt ctgctggcct ctcagaaacc aaatagttgc
1561 ctctctggtc accaaactcc aatcaatcac cagccggcaa aaggaaagaa aggtttcaga
1621 gcctgtgtgt tctttctctg gatttactct tcagttcctc ttttggtttg tttggttggt
1681 ttttttggc cacgtatagt atatttaagg atcaaatgtg gcatattcat tctagctaag
1741 tccttgaaag caggaaaatg ctcatgaaag gactgtcctt gccccaaggt gcctcttctt
1801 ctctagtact agacactcag ggtcagcctg agatttcaag aggctacagc ctgaccaggc
1861 cgtcttctta ttacccagca ggctgtgtgc atgcaaaccc aaagacatat atgcacatct
1921 gtgtggtatt tcagcatgtc tctgtccaat gtttgatatg ttaacatttg aatttaatgc
1981 tgtcctcctt atgggtttct accaaagaga aaccagccac ttatcaattt tagtttcttg
2041 ctgagctgcc agaaagtatt acagagaagc acatccaagc tgtctgtggc ctacgcctgc
2101 agggggtggg gggcctgaat ctccttggcc ttcagttcca cctccacctc tggctttagg
2161 gtctccagct gttgcctgag tagtagcttt gattacagcg gtaaagtcct ccaacttgga
2221 gtcctttctg gtgggaagca tggtctgctc gcagcacagc actgagcaga cccgtgggcc
2281 tgacttccct ggtgacttca gtgccttttt gtttgcagag aaaagagtgg ggcactttgc
2341 ttgaagctct ctgctggctt gcccctggca ggaagtggac aatggtgcta tagagccaag
2401 gacacagcct cagagcacag ggtgattgat gatcagcctc tttcccatca agcttcccgg
2461 tcaggctttg actttgaaga tgcgaggtta ctagactgca ttgacagcat cagattatga
2521 ctccaactct tgagtagttc agacttaaaa ccaatcagcc agagtagcca ggactgcaaa
2581 gacactcaat acagatggag aaaaacttgt ccctttaaaa gagggccagt gtttcaattg
2641 agcctccaga ggagaccact ttcatgttgt gcttgccttt ccataccctt tcctcgggtt
2701 gttttaagcc caagcttctc cgtgtagcct aaaaagttcc ctaccagccc agctgaagcc
2761 acactgctcc cgtcccagaa gaacgccaaa tccttgtcat tcaaactgtg catcgtttgc
2821 agagctgcaa aaagcaacat gagctagcga ctctgaggtt gtgcacgcca tcagcccctt
2881 ggctgcctga ggtctcatgc ccagccttac acctctctcc cttaagaagc ccccgtcctg
2941 ctgtgtacta caggggcacg tggaatcatt cccttcatcc tgcatgtctg tagcgttagg
3001 agaaggcatg gctcctgc
```

MASPAIGQRP YPLLLDPEPP RYLQSLGGTE PPPPARPRRC IPTALIPAAG ASEDRGGRRS
GRRDPEPTPR DCRHARPVRP GLQPRLRLRP GSHRPRDVRS IFEQPQDPRV LAERGEGHRF
VELALRGGPG WCDLCGREVL RQALRCANCK FTCHSECRSL IQLDCRQKGG PALDRRSPGS
TLTPTLNQNV CKAVEETQHP PTIQEIKQKI DSYNSREKHC LGMKLSEDGT YTGFIKVHLK
LRRPVTVPAG SGPSPSMDAI KEVNPAATTD KRTSFYLPLD AIKQLHISST TTVSEVIQGL
LKKFMVVDNP QKFALFKRIH KDGQVLFQKL SIADYPLYLR LLAGPDTDVL SFVLKENETG
EVEWDAFSIP ELQNFLTILE KEEQDKIHQL QKKYNKFRQK LEEALRESQG KPG

VHL **GenBank Accession No. NM_000551**

Nucleic acids: SEQ ID NO: 129 (coding 2145-855); Amino acids

```
   1 cctcgcctcc gttacaacgg cctacggtgc tggaggatcc ttctgcgcac gcgcacagcc
  61 tccggccggc tatttccgcg agcgcgttcc atcctctacc gagcgcgcgc gaagactacg
 121 gaggtcgact cgggagcgcg cacgcagctc cgccccgcgt ccgacccgcg gatcccgcgg
 181 cgtccggccc gggtggtctg gatcgcggag ggaatgcccc ggagggcgga gaactgggac
 241 gaggccgagg taggcgcgga ggaggcaggc gtcgaagagt acggccctga agaagacggc
 301 ggggaggagt cgggcgccga ggagtccggc ccggaagagt ccggcccgga ggaactgggc
 361 gccgaggagg agatggaggc cgggcggccg cggcccgtgc tgcgctcggt gaactcgcgc
 421 gagccctccc aggtcatctt ctgcaatcgc agtccgcgcg tcgtgctgcc cgtatggctc
 481 aacttcgacg gcgagccgca gccctaccca acgctgccgc ctggcacggg ccgccgcatc
 541 cacagctacc gaggtcacct ttggctcttc agagatgcag ggacacacga tgggcttctg
 601 gttaaccaaa ctgaattatt tgtgccatct ctcaatgttg acggacagcc tatttttgcc
 661 aatatcacac tgccagtgta tactctgaaa gagcgatgcc tccaggttgt ccggagccta
 721 gtcaagcctg agaattacag gagactggac atcgtcaggt cgctctacga agatctggaa
 781 gaccacccaa atgtgcagaa agacctggag cggctgacac aggagcgcat tgcacatcaa
 841 cggatgggag attgaagatt tctgttgaaa cttacactgt ttcatctcag cttttgatgg
 901 tactgatgag tcttgatcta gatacaggac tggttccttc cttagtttca aagtgtctca
 961 ttctcagagt aaaataggca ccattgctta aaagaaagtt aactgacttc actaggcatt
1021 gtgatgttta ggggcaaaca tcacaaaatg taatttaatg cctgcccatt agagaagtat
1081 ttatcaggag aaggtggtgg catttttgct tcctagtaag tcaggacagc ttgtatgtaa
1141 ggaggtttgt ataagtaatt cagtgggaat tgcagcatat cgtttaattt taagaaggca
1201 ttggcatctg cttttaatgg atgtataata catccattct acatccgtag cggttggtga
1261 cttgtctgcc tcctgctttg ggaagactga ggcatccgtg aggcagggac aagtctttct
1321 cctctttgag accccagtgc ctgcacatca tgagccttca gtcagggttt gtcagaggaa
1381 caaaccaggg gacactttgt tagaaagtgc ttagaggttc tgcctctatt tttgttgggg
1441 ggtgggagag gggaccttaa aatgtgtaca gtgaacaaat gtcttaaagg gaatcatttt
1501 tgtaggaagc atttttata attttctaag tcgtgcactt tctcggtcca ctcttgttga
1561 agtgctgttt tattactgtt tctaaactag gattgacatt ctacagttgt gataatagca
1621 tttttgtaac ttgccatccg cacagaaaat acgagaaaat ctgcatgttt gattatagta
1681 ttaatggaca aataagtttt tgctaaatgt gagtatttct gttccttttt gtaaatatgt
1741 gacattcctg attgatttgg gttttttgt tgttgttgtt ttgttttgtt ttgttttttt
1801 gagatggagt ctcactcttg tcacccaggc tggagtgcag tggcgccatc tcggctcact
1861 gcaacctctg cctcctgggt tcacgtaatc ctcctgagta gctgggatta caggcgcctg
1921 ccaccacgct ggccaatttt tgtactttta gtagagacag tgtttcgcca tgttggccag
1981 gctggtttca aactcctgac ctcaggtgat ccgcccacct cagcctccca aaatggtggg
2041 attacaggtg tgtgggccac cgtgcctggc tgattcagca ttttttatca ggcaggacca
2101 ggtggcactt ccacctccag cctctggtcc taccaatgga ttcatggagt agcctggact
2161 gtttcatagt tttctaaatg tacaaattct tataggctag acttagattc attaactcaa
2221 attcaatgct tctatcagac tcagtttttt gtaactaata gatttttttt tccacttttg
2281 ttctactcct tccctaatag ctttttaaaa aaatctcccc agtagagaaa catttggaaa
2341 agacagaaaa ctaaaaagga agaaaaaaga tccctattag atacacttct taaatacaat
2401 cacattaaca ttttgagcta tttccttcca gccttttag ggcagatttt ggttggtttt
2461 tacatagttg agattgtact gttcatacag ttttataccc tttttcattt aactttataa
2521 cttaaatatt gctctatgtt agtataagct tttcacaaac attagtatag tctccctttt
2581 ataattaatg tttgtgggta tttcttggca tgcatcttta attccttatc ctagcctttg
2641 ggcacaattc ctgtgctcaa aaatgagagt gacggctggc atggtggctc ccgcctgtaa
2701 tcccagtact ttgggaagcc aaggtaagag gattgcttga gcccagaact tcaagatgag
```

```
2761 cctgggctca tagtgagaac ccatctatac aaaaaatttt taaaaattag catggcggca
2821 cacatctgta atcctagcta cttggcaggc tgaggtgaga agatcattgg agtttaggaa
2881 ttggaggctg cagtgagcca tgagtatgcc actgcactcc agcctggggg acagagcaag
2941 accctgcctc aaaaaaaaaa aaaaaaaa
```

```
MPRRAENWDE AEVGAEEAGV EEYGPEEDGG EESGAEESGP EESGPEELGA EEEMEAGRPR
PVLRSVNSRE PSQVIFCNRS PRVVLPVWLN FDGEPQPYPT LPPGTGRRIH SYRGHLWLFR
DAGTHDGLLV NQTELFVPSL NVDGQPIFAN ITLPVYTLKE RCLQVVRSLV KPENYRRLDI
VRSLYEDLED HPNVQKDLER LTQERIAHQR MGD
```

Experimental procedures

Family with CC-RCC and t(1;3)(q32.1;q13.3), paired CC-RCC tumors/normal kidney tissues, and cell lines

The clinical and genetic details of the Japanese kindred with familial CC-RCC have been previously published (Kanayama *et al.*, 2001). The EBV-transformed lymphoblastoid cell lines FRCC3 and FRCC5 used in this study were established from two affected translocation carriers.

Four tumors were from three members of the t(1;3) family, and 53 matched pairs of CC-RCC were collected from the University of Tokushima in Japan.

Nine established RCC cell lines were purchased from ATCC: A-498, A-704, Caki-1, Caki-2, SW-839, ACHN, 786-O, 769-P, and SW-156.

Construction of BAC contigs and FISH analyses

Forty-four 1q32.1 and 3q13.3 BAC clones were obtained from the BACPAC Resource Center (Children's Hospital, Oakland Research Institute) or ResGen Invitrogen Corporation. The clones were selected based on information in the BAC clone mapping databases and Human Genome Sequence Draft database. The details of the BACs are listed in the Supplemental Experimental Procedures.

Standard dual-color FISH was performed by hybridizing each of the 44 BAC clones to metaphase slides prepared from FRCC3 or FRCC5. In all hybridizations, the PAC clone 160H23 from the 1q subtelomere (Cytocell Ltd, UK) was included as a marker of the normal chromosome 1 and the der(3) chromosome.

Long-range PCR, Southern blot analysis, and Northern blot analysis

Long-range PCR was used for the amplification of the breakpoints and the generation of DNA probes for Southern blot analysis with an Advantage Genomic PCR kit (Clontech, USA). PCR was carried out following the manufacturer's user manual. Southern blot and Northern blot analyses were performed following the standard protocol. Human multiple tissue Northern blots

were purchased from Clontech (Cat. #7780-1). Details of these analyses can be found in the Supplemental Experimental Procedures.

Mutation analysis

Mutation analysis of *LSAMP, NORE1A*, and *NORE1B* was performed in the 53 sporadic CC-RCCs and 9 RCC cell lines. Each exon of *LSAMP, NORE1A*, and *NORE1B* was amplified by PCR using primers derived from the flanking intronic or UTR sequences (see Table A in the Supplemental Data). The PCR products were then purified and subjected to direct DNA sequencing using PE Applied Biosystems.

Real-time quantitative RT-PCR

Total RNA from normal kidney tissues, RCC cell lines, and sporadic tumors was subjected to real-time quantitative PCR using an ABI PRISM 7700 Sequence Detection System. Specific primer and probe were designed for *LSAMP* and *NORE1A* using Primer Express v1.5a (Applied Biosystems). The primer sequences and the details of the real-time RT-PCR analysis are described in the Supplemental Experimental Procedures.

DNA methylation analysis and demethylation treatment by 5-aza-2'-deoxycytidine (5-aza-CdR)

Methylation analysis was performed for the promoter CpG islands of *LSAMP* and *NORE1A*. Bisulfite-PCR followed by restriction enzyme digestion analysis was used. Eight RCC cell lines were demethylated by 5-aza-CdR (Sigma, USA) treatment. The primers and the details of the analyses are given in the Supplemental Experimental Procedures.

LOH analysis

LOH detection for *LSAMP* and *NORE1* was performed by genotyping the 53 paired normal/tumor DNA samples. The microsatellite markers flanking the *LSAMP* locus are *D3S3681, D3S1271, D3S1267*, and *D3S1292*. *NORE1* locus markers include *D1S413* and *D1S249*. All the markers were obtained from ABI Prism Linkage Mapping Set version 2, panel 1 and 2 (Applied Biosystems). The details of LOH analysis are described in the supplemental experimental procedures.

Cell growth assay

Expression plasmids *pEGFP-LSAMP, -NORE1A*, and *-Nore1* were generated by ligating cDNAs of *LSAMP, NORE1A*, and murine *Nore1* to N- or C-terminal enhanced green fluorescent protein vectors (*pEGFP-N1/-C1*) (Clontech, USA). Expression plasmids were microinjected and transfected into two RCC cell lines, A-498 and/or Caki-1, for cell growth-suppression assay.

Inducible experiments and nuclear fractionation assays were also performed for the nuclear location of Norel. Detailed methods are provided in the Supplemental Data.

URLs. University of California, Santa Cruz (UCSC) Human Genome Browser at the web site: www.genome.ucsc.edu; Discovery System Human Genome Browser: at the web site: www.cds.celera.com; Human Genome Browser NCBI: at the web site ncbi.nlm.nih.gov.

Supplemental Experimental Procedures

BAC clones used for construction of BAC contigs and FISH analyses

The 20 BAC clones from 1q32.1 are RP11-196B7, RP11-70G20, RP11-219P13, RP11-45F21, RP11-104A2, RP11-124A11, RP11-149C8, RP11-237N7, RP11-142B3, RP11-421E17, RP11-54L22, RP11-262N9, RP11-237C22, RP11-145I13, RP11-57I17, RP11-534L20, CTD-2245C1, CTD-2321B11, CTD-2278G17, and RP11-343H5.

The 24 BAC clones from 3q13.3 are RP11-138N21, RP11-58D2, RP11-324H4, RP11-165B13, RP4-635B5, RP11-484M3, RP11-829I14, RP11-641I23, RP11-643A3, RP11-891J4, RP11-281N16, RP11-50N14, RP11-728O20, RP11-899P8, RP11-716E6, RP11-1115L2, RP11-60P15, RP11-47C16, CTD-2246M24, RP11-149B11, CTD-2514L8, CTD-2016D14, CTC-804P8, and CTC-2006J5.

Long-range PCR, Southern blot analysis, and Northern blot analysis

Long-range PCR was used for the amplification of the breakpoints and the generation of DNA probes for Southern blot analysis with an Advantage Genomic PCR kit (K1906-Y, CLONTECH Laboratories, Inc., USA). PCR was carried out following the manufacturer's user manual. BAC clones spanning the 1q32.1 (RP11-54L22) and 3q13.3 (RP11-281N16) breakpoints were used as PCR templates. Four approximately 10-kb and five 4- to 6-kb DNA probes were synthesized for 1q breakpoint mapping and six 5- to7- kb DNA probes were generated for Southern blot analysis in 3q breakpoint mapping. Two EBV-transformed lymphoblastoid cell lines of two patients from the t(1q;3q) family and two normal EBV-transformed lymphoblastoid cell lines were used for Southern blot analysis. Fifteen microgram aliquots of genomic DNA were digested using *Bam*HI, *Eco*RI, *Hin*dIII, *Stu*I, *Eco*RV, *Xba*I, *Bgl*I, and *Bgl*II. Completely digested DNA samples were separated by size on a 0.8% agarose in 1 × TBE buffer. Southern blot to nylon membrane and subsequent hybridization were performed following the standard protocol.

For Northern blot analysis, human multiple tissue Northern blots were purchased from Clontech (USA, Cat. #7780-1). Northern blots were also prepared with RNA from normal

kidney (Clontech) and with RNA extracted from the nine RCC cell lines, the EBV lines FRCC3 and FRCC5, two EBV lines from normal individuals, and normal kidney tissues from two patients with sporadic CC-RCC. Total RNA was extracted using the Trizol Reagent kit (Invitrogen), and 15 μg of total RNA of each sample was for the Northern blots. Probes specific for *NORE1A* (exon 1α), *NORE1B* (exon 2β), *LSAMP* (exon 1), and β-actin were synthesized by PCR labeled with α-^{32}P and hybridized to the Northern filters under stringent conditions.

RT-PCR

RT-PCR was performed using 5 μg of total RNA isolated from the nine RCC cell lines and nine normal kidney tissues, Superscript-II RT (Invitrogen), random hexamer primers, and specific primer pairs. Specific primers from *LSAMP* and *NORE1A* were used for fusion transcripts analysis. The primer sequences are given in Table A of supplemental data online. PCR was carried out at 95°C for 5 min, followed by 95°C for 30 s, 58°C for 30 s and 72°C for 45 s, for 35 cycles.

LOH analysis

PCR was performed according to the manufacturer's protocol. For each individual, 1 μl of PCR product from each marker was then pooled. One microliter of this mixture was added to 10 μL of Hi-Di formamide and 0.1 μl of ROX 400HD size standard and denatured at 95 °C for 5 min before loading the samples into an ABI Prism 3700 DNA Analyzer (Applied Biosystems). Analysis of raw data and assessment of LOH were performed with Genescan v. 3.7 and Genotyper v. 3.7 software, respectively (Applied Biosystems). LOH was defined according to the following formula: LOH index = (T2/T1)/(N2/N1), where T was the tumour sample, N was the matched normal sample, and 1 and 2 were the intensities of smaller and larger alleles, respectively. If the ratio was less than 0.67 or more than 1.3, the result was determined to be LOH.

Real-time quantitative RT-PCR

Two micrograms of total RNA from 9 normal kidney tissues, the 9 RCC cell lines, and 16 sporadic tumors with *LSAMP* and/or *NORE1A* promoter methylation (RNA from other tumors was not available for analysis) were reverse-transcribed in a 100 μl reaction mixture using MultiScribe Reverse Transcriptase following the manufacturer's instruction (Applied Biosystems). Real-time quantitative PCR was performed using an ABI PRISM 7700 Sequence Detection System. Specific primer and probe sequences were designed for *LSAMP* and *NORE1A* using Primer Express v1.5a (Applied Biosystems).

LSAMP forward primer: 5'-CAATGGCCGTCCTGAACCT-3' (SEQ ID NO:106);
LSAMP reverse primer: 5'-CAAATTCCCTTCCAGTTGGTGTA-3' (SEQ ID NO:107);
LSAMP Taqman probe: 5'-6FAM-TTATCACCTGGAGACACC-MGBNFQ (SEQ ID NO:108);
NORE1A forward primer: 5'-GCGCTGCACTAACTGTAAATTCA-3' (SEQ ID NO:109);
NORE1A reverse primer: 5'-GGGATAAACCCTCCTGCTGACT-3' (SEQ ID NO:110);
NORE1A taqman probe: 5'-6FAM-TCACCCAGAATGCCGCA-MGBNFQ-3' (SEQ ID NO:111).

Of each sample, 100 ng was amplified using the following PCR conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. A series of five 1:2 dilutions of pooled normal sample served as a standard curve for relative quantification. Each tumor sample was normalized to an endogenous control, β-actin, and then normalized to the standard curve. Reported are values of fold change from pooled normal.

DNA methylation analysis

Two μg of genomic DNA from each cell line, tumor and normal kidney tissue was denatured in 0.3 M NaOH for 15 min at 37°C. Cytosines were sulfonated in 5 mM hydroquinone (Sigma) and 3.12 M sodium bisulfite (Sigma) for 16 h at 50°C. The DNA samples were desalted through columns, desulfonated in 0.3 M NaOH and precipitated with ethanol. DNA sequences were amplified by nested PCR. Approximately 50 ng of bisulfite-treated DNA was firstly amplified in a reaction volume of 30 μl with respective outer primer pairs:
LSAMP-BISF-OF (5'-TGGTAGAGGAGTATTTAGTTATAGAGAGA-3') (SEQ ID NO:112),
LSAMP-BISF-OR1 (5'-TCTCAATAAAACCAATAACAACTATTTC-3') (SEQ ID NO:113),
NORE1A-BISF-OF2 (5'-AAGAGGTAGGGTTGAAGGTTTAGGGTTT-3') (SEQ ID NO:114), and
NORE1A-BISF-OR2 (5'-CTCRAAACCRCTCAAACTCTATAAATAAC-3') (SEQ ID NO:115).
PCR was carried out at 95°C for 8 min, followed by 95°C for 30 s, 58°C for 30 s and 72°C for 1 min, for 30 cycles. A nested PCR was performed using 1 μl of the initially amplified products and the respective internal primer pairs:
LSAMP-BISF-IF (5'-TGTTTGGGTTTTATGAGGGTTTTGT-3') (SEQ ID NO:116) and
LSAMP-BISF-IR (5'-CRACTAAACTCTCCTAACCATAATAACCAC-3') (SEQ ID NO:117),
NORE1A-BISF-IF2 (5'-GAATTTTGTAGTTGTTTTAGGTGAAGA-3') (SEQ ID NO:118), and
NORE1A-IR2 (5'-CRACRACTCRAAATCCAATAATAA-3') (SEQ ID NO:119)
with similar conditions as described for the preceding PCR amplification. The PCR products were purified using Microcon YM-100 (Millipore Corporation, USA). For isoform *NORE1B*, methylation analysis was performed as described in Tommasi *et al.* 2002. For restriction enzyme

analysis of PCR products from bisulfite-treated DNA, 30 ng of the PCR products was digested with 10 units of *Taq*I (Invitrogen, USA) and separated by size on a 2.0% TAE gel.

Demethylation of LSAMP and NORE1A by 5-aza-2'-deoxycytidine (5-aza-CdR) treatment

Eight RCC cell lines (except the slow-growing A-704 cell line) were subjected to 5-aza-CdR (Sigma Chemical Co., St. Louis, MO) treatment. Approximately 5×10^5 cells for each line were seeded on a 100-mm plate and incubated for 24 h. The cells were cultured up to 14 d in complete media which contained 2.5 μM of 5-aza-CdR, and media was changed at 2-d intervals. Isolated total cellular RNAs from RCC cell lines treated and untreated with 5-aza-CdR were analyzed with real-time quantitative RT-PCR.

Cell growth assay

RCC cell lines A-498 and/or Caki-1, growing logarithmically on glass coverslips and maintained in 10% fetal calf serum, were microinjected or lipid-mediated transfection with *pEGFP-LSAMP*, *pEGFP-NORE1A*, *pEGFP-Nore1* or the vector control *pEGFP-C1/pEGFP-N1* (50 ng/ml). Two hours after injection or 24 h after lipid-mediated transfection (Lipofectamine2000 reagents, Invitrogen), EGFP/EGFP-Nore1-expressing cells were fixed and stained with Texas Red-labeled phalloidin to reveal F-actin architecture and Hoechst 33342 (blue) to visualize DNA (nuclei). To monitor proliferation in cells expressing EGFP-LSAMP, EGFP-NORE1A, and Nore1 fusion proteins or EGFP, 40-60 cells were microinjected with expression plasmids for the indicated protein, and returned to the incubator for 2 h. The number of successfully injected/expressing cells were then counted on an inverted epifluorescence microscope and thereafter at the selected times.

Supplemental inducible experiments and nuclear fractionation assay for nuclear location of Nore1

To confirm the nuclear location of Nore1, me performed experiments by cloning Nore1 into the pIND(SP1)-Hygro vector (Invitrogen, Carlsbad, CA) and transfecting the plasmid into 293-T cells using Lipofectamine2000 reagents (Invitrogen). After selection in hygromycin, cell populations were pooled at an early passage and assayed for the effects of Nore1 induction on cell proliferation by growth curve analysis. Selected cells were plated at a cell density of 2.5 x 10^5 cells/well in triplicate and induced with Ponasterone A 24 h later. Cells were counted using a Coulter counter every 24 h. Western analysis was performed on lysates at each time point.

For nuclear fractionation assay, 293-T cells were transfected with 1 μg of pcDNAFlag-*Nore1* using CaPO4 (Invitrogen, Carlsbad CA). Forty-eight hours later cells were harvested and

processed for subcellular fractionation. Protein determinations were made for each fraction and equivalent amounts were loaded on to gel. The cytoplasmic, membrane and nuclear fractions were then subjected to Western analysis using an anti-FLAG antibody (Sigma, St. Louis. MO).

References for Examples

Bodmer, D *et al.*, (2002a). Disruption of a novel MFS transporter gene, DIRC2, by a familial renal cell carcinoma-associated t(2;3)(q35;q21). Hum. Mol. Genet. *11*, 641-649.

Bodmer, D. *et al.*, (2002b). Cytogenetic and molecular analysis of early stage renal cell carcinomas in a family with a translocation (2;3)(q35;q21). Cancer Genet. Cytogenet. *134*, 6-12.

Bodmer, D. *et al.*, (1998). An alternative route for multistep tumorigenesis in a novel case of hereditary renal cell cancer and a t(2;3)(q35;q21) chromosome translocation. Am. J. Hum. Genet. *62*, 1475-1483.

Bodmer, D., *et al.* (2002c). Understanding familial and non-familial renal cell cancer. Hum. Mol Genet. *11*, 2489-2498.

Carpten, J. D. *et al.*, Nat. Genet. *32*, 676-680.

Cohen, A.J. *et al.* (1979). Hereditary renal-cell carcinoma associated with a chromosomal translocation. N. Engl. J. Med. *301*, 592-595.

Dammann, R. *et al.* (2000). Epigenetic inactivation of a RAS association domain family protein from the lung tumor suppressor locus 3p21.3. Nat. Genet. *25*, 315-319.

Dreijerink, K *et al.* (2001). The candidate tumor suppressor gene, RASSF1A, from human chromosome 3p21.3 is involved in kidney tumorigenesis. Proc. Natl. Acad. Sci. USA *98*, 7504-7509.

Druck, T. *et al.* (1997). Structure and expression of the human FHIT gene in normal and tumor cells. Cancer Res *57*, 504-512.

Druck, T. *et al.* (2001). The DIRC1 gene at chromosome 2q33 spans a familial RCC-associated t(2;3)(q33;q21) chromosome translocation. J. Hum. Genet. *46*, 583-589.

Eleveld, M.J. *et al.* (2001). Molecular analysis of a familial case of renal cell cancer and a t(3;6)(q12;q15). Genes Chromosomes Cancer *31*, 23-32.

Gemmill, R.M. *et al.*, (2002). The TRC8 hereditary kidney cancer gene suppresses growth and functions with VHL in a common pathway. Oncogene *21*, 3507-3516.

Hesson, L. *et al.* (2003). NORE1A, a homologue of RASSF1A tumor suppressor gene is inactivated in human cancers. Oncogene *22*, 947-954.

Jones, P.A. *et al.* (2002). The fundamental role of epigenetic events in cancer. Nat. Rev. Genet. *3*, 415-428.

Kanayama, H. *et al.* (2001). Association of a novel constitutional translocation t(1q;3q) with familial renal cell carcinoma, J. Med. Genet. *38*, 165-170.

Khokhlatchev, A. *et al.* Curr. Biol. *12*, 253-265.

Koolen, M.I. *et al.* (1998). A familial case of renal cell carcinoma and a t(2;3) chromosome translocation. Kidney Int. *53*, 273-275.

Kovacs, G. *et al.*. (1989). Tissue-specific expression of a constitutional 3;6 translocation: development of multiple bilateral renal-cell carcinomas. Int. J. Cancer *43*, 422-427.

Kovacs, G. *et al.* (1988). Loss of der(3) in renal carcinoma cells of a patient with constitutional t(3;12). Hum Genet *78*, 148-150.

Latif, F. *et al.*. (1993). Identification of the von Hippel-Lindau disease tumor suppressor gene. Science *260*, 1317-1320.

Morrissey, C *et al.*. (2001). Epigenetic inactivation of the RASSF1A 3p21.3 tumor suppressor gene in both clear cell and papillary renal cell carcinoma. Cancer Res. *61*, 7277-7281.

Nickerson, M.L. *et al.* (2002). Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. Cancer Cell *2*, 157-164.

Ohta, M. *et al.* (1996). The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell *84*, 587-597.

Ortiz-Vega, S. *et al.* (2002). The putative tumor suppressor RASSF1A homodimerizes and heterodimerizes with the Ras-GTP binding protein Norel. Oncogene *21*, 1381-1390.

Pimenta, A.F. *et al.*. (1996). cDNA cloning and structural analysis of the human limbic-system-associated membrane protein (LAMP). Gene *170*, 189-195.

Podolski, J. *et al.* (2001). Characterization of a familial RCC-associated t(2;3)(q33;q21) chromosome translocation. J. Hum. Genet. *46*, 685-693.

Schmidt, L. *et al.* (1995). Mechanism of tumorigenesis of renal carcinomas associated with the constitutional chromosome 3;8 translocation. Cancer J. Sci. Am. *1*, 191.

Sellar, G. C. *et al.* (2003). OPCML at 11q25 is epigenetically inactivated and has tumor-suppressor function in epithelial ovarian cancer. Nat. Genet. *34*, 337-343.

Siprashvili, Z. *et al.* (1997). Replacement of Fhit in cancer cells suppresses tumorigenicity. Proc Natl Acad Sci U S A *94*, 13771-13776.

Storkel, S *et al.* (1997). Classification of renal cell carcinoma: Workgroup No. 1. Union Internationale Contre le Cancer (UICC) and the American Joint Committee on Cancer (AJCC). Cancer *80*, 987-989.

Teh, B.T. *et al.* (1997). Familial non-VHL non-papillary clear-cell renal cancer. Lancet *349*, 848-849.

Tomlinson, I.P. *et al.* (2002). Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat. Genet. *30*, 406-410.

Tommasi, S. *et al.* (2002). RASSF3 and NORE1: identification and cloning of two human homologues of the putative tumor suppressor gene RASSF1. Oncogene *21*, 2713-2720.

van den Berg, A. *et al.* (1997). Involvement of multiple loci on chromosome 3 in renal cell cancer development. Genes Chromosomes Cancer *19*, 59-76.

van Kessel, A.G. *et al.* (1999). Renal cell cancer: chromosome 3 translocations as risk factors. J. Natl. Cancer Inst. *91*, 1159-1160.

Vavvas, D . *et al.* (1998). Identification of Norel as a potential Ras effector. J. Biol. Chem. *273*, 5439-5442.

Vos, M.D. *et al.* (2000). Ras uses the novel tumor suppressor RASSF1 as an effector to mediate apoptosis. J. Biol. Chem. *275*, 35669-35672.

Woodward, E.R *et al.* (2000). Familial clear cell renal cell carcinoma (FCRC): clinical features and mutation analysis of the VHL, MET, and CUL2 candidate genes. J. Med. Genet. *37*, 348-353.

Yoon, J.H. *et al.*. (2001). Hypermethylation of the CpG island of the RASSF1A gene in ovarian and renal cell carcinomas. Int. J. Cancer *94*, 212-217.

Zbar, B. *et al.* (2003). Studying cancer families to identify kidney cancer genes. Annu. Rev. Med. *54*, 217-233.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make changes and modifications of the invention to adapt it to various usage and conditions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The preceding preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The entire disclosure of all applications, patents and publications, cited above and in the figures are hereby incorporated in their entirety by reference.